"ROLE OF RALOXIFENE IN PREVENTION AND TREATMENT OF POST MENOPAUSAL OSTEOPOROSIS"

DISSERTATION

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VIJAY LAKSHMI SOOD

CERTIFICATE

This is to certify that the work entitled **"ROLE OF RALOXIFENE IN PREVENTION AND TREATMENT OF POST MENOPAUSAL OSTEOPOROSIS"** which is being submitted as a thesis for M.S., (Obstetrics & Gynaecology) examination, 2004, Bundelkhand University by Dr. Vijay Lakshmi, has been carried out in the department of Obstetrics & Gynaecology, M.L.B. Medical College, Jhansi, under my direct supervision and guidance. The observations recorded have been checked and verified by me from time to time.

She has put in the necessary stay in the department as required by the regulations of Bundelkhand University.

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A Word of Gratitude

I am overwhelmed at this opportunity of expressing my gratitude towards my esteemed teacher and guide

DR. (MRS.) MRIDULA KAPOOR

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A scintillating personality, perfectly blended with qualities of a good surgeon. An adorable teacher, possessing a rare enthusiasm and quest for knowledge, who always strives to excel.

I feel heartedly indebted for the precious guidance, generosity and affection she has bestowed upon me, from beginning to the end of this work. Despite her busy schedule and numerous responsibilities, she found time to extend her help and support to complete this study.

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VIJAY LAKSHMI SOOD

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Last but most of all, I thank my study patients, who taught me all that I can ever hope to learn.

V. Sood

VIJAY LAKSHMI SOOD

DEDICATED

TO

MY HUSBAND

CONTENTS

INTRODUCTION

In her lifetime, a woman is overtly exposed to various hormones, which play a major role in determining her physical and mental well being. Every phase of a woman's life is marked by certain hormonal changes taking place within her body. Menopause is one such phase, which by far is the most crucial stage of a woman's life.

Menopause, whether natural or artificial, marks the end of the childbearing years of a woman. Thanks to improved nutrition and disease control, today's woman spends one third of her life in the postmenopausal stage. It therefore becomes important to cease all pain and discomfort from the postmenopausal days and enable her to enjoy a healthy life beyond the fertile span.

In the postmenopausal phase, there is an increased risk of developing osteoporosis and cardiovascular complications. As per published data, over 61 million Indians have osteoporosis and 80% are women. It has been estimated that 35% of postmenopausal women in India are osteoporotic resulting in a high incidence of vertebral and non-vertebral (hip, wrist) fractures in elderly women.

Osteoporosis is basically a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. Osteoporotic fractures, particularly of the hip, result in considerable

morbidity including pain, impaired quality of life, functional decline and associated hospitalization and lengthy nursing home or rehabilitation center stays. Furthermore a 1.5 to 3 – fold increase in mortality has been associated with the occurrence of any major osteoporotic fracture.

The postmenopausal bone loss is primarily caused by estrogen deficiency. Osteoclast activity is promoted by a drop in estrogen levels which leads to reduced transforming growth factor-beta (TGF-β) mediated osteoclast apoptosis, the latter actually being controlled by estrogen.

Reduced D-hormone synthesis in the kidney, due to decreased activity of 1 α- hydroxylase enzyme, which in turn is caused by estrogen deficiency, is also a pathogenetic co-factor in postmenopausal osteoporosis. A deficiency in the number and activity of vitamin D receptors in the intestines and bones is evident, which too is dependent on estrogen deficiency.

As a consequence of all of the above, the bone trabeculae become thinner which frequently results in perforation of, predominantly the horizontal trabeculae and leads to a reduction in connectivity of bone architecture and thus in bone quality. In this way, the increased vertebral and radial fracture rates in postmenopausal women are easily understood. Antiresorptive agents like bisphosphonates increase bone mineral density and reduce the incidence of osteoporotic fractures. But

because of poor bioavailability and intolerable gastrointestinal side effects, patient compliance is very poor. Hormone replacement therapy (HRT) is used world wide to reduce the symptoms associated with menopause. But since the conventional hormone replacement therapy (HRT) regimen causes considerable adverse effects on the body (e.g. an increased risk of breast and uterine cancer), the need for a multiaction enhanced HRT module was strongly felt. This is where the advent of Selective Estrogen Receptor Modulators (SERM) came into limelight. The SERMs enabled desired HRT action at required areas like bone and CVS and inhibited adverse actions at the breast and uterus.

The action of SERMs such as raloxifene are tissue selective and cannot be definitely described as either estrogen receptor agonists or antagonists. They partially mimic the effect of estrogen on bone and cardiovascular system while functioning as antiestrogen in the breast tissue.

Raloxifene in particular has been shown to not only increase bone density and favorably affect the lipid profile but also to reduce the risk of breast and uterine cancer.

The present study aims to test the efficacy of raloxifene in prevention and treatment of osteoporosis by measuring bone mineral density (BMD) changes.

AIMS
&
OBJECTIVES

The aims and objectives of the present study are:-

1. To evaluate the efficacy of Raloxifene in the treatment of postmenopausal (surgical or natural) osteoporosis, taking into consideration the following parameters:

a) Bone Mineral Density.

b) Serum Calcium levels.

2. To assess the role of Raloxifene in the prevention of postmenopausal (surgical or natural) osteoporosis.

3. To evaluate the side effects of Raloxifene when prescribed in dosages intended to treat/prevent osteoporosis.

REVIEW OF LITERATURE

Osteoporosis

Osteoporosis in the term used for diseases that cause a reduction in the mass of bone per unit volume. It is used to define any degree of skeletal fragility sufficient to increase the risk of fracture. The reduction in mass results from an imbalance in the processes that influence the acquisition and maintenance of skeletal mass and is not accompanied by a change in the ratio of the mineral phase to the organic phase or by any abnormality in bone mineral or organic matrix. Histologically, the disorder is characterized by a decrease in cortical thickness and in the number and size of the trabeculae of cancellous bone. Individual trabecular plates are abnormally perforated and may be fractured, and trabecular connectivity is reduced.

Bone Turnover and Remodeling

The remodeling of bone (its formation and resorption) is a continuous process. Since the bone mass is decreased in osteoporosis, the affected individual either failed to attain optimal skeletal mass during the first 3 decades of life, and/or the rate of bone resorption exceeded that of bone formation after peak skeletal mass was attained. Bone formation is higher in cortical than in cancellous bone. This difference is exaggerated by the normal menopause and exaggerated further in patients with osteoporosis, because rates of formation of cancellous

bone tend to be lower in patients with osteoporosis, particularly in women after the menopause.

Age and Menopause as Risk Factors for Osteoporosis

After age 40 to 50, cortical bone is lost at a rate of about 0.3 to 0.5 percent per year in both sexes. Around the menopause in women an accelerated loss of cortical bone is superimposed on the age-related loss. Loss of trabecular bone begins at an earlier age in both sexes but is probably greater in women. The cumulative losses of bone mass range from 20 to 30 percent in men and 40 to 50 percent in some women. Bone loss involves predominantly trabecular bone in the spine and distal radius in women and the spine and hip in both women and men.

The age-related loss of bone begins earlier and proceeds more rapidly in women and tends to accelerate before the menopause.

Other Risk Factors

The facts that accelerated bone loss accompanies the menopause in some women and that premature osteoporosis occurs after premature surgical menopause suggest that estrogens play a major role in preventing bone loss. Osteoporotic women also have a higher incidence of cigarette smoking which might affect bone remodeling directly or have secondary effects on ovarian function. Excessive alcohol consumption can decrease bone formation and is a risk factor for osteoporosis.

Dietary calcium intake during the first 3 decades of life influences the ultimate peak bone mass, and calcium intake during adult life also has a small effect on bone mass and risk of fracture. Inability to synthesize adequate amounts of 1, 25-dihydroxyvitamin D [1,25$(OH)_2$D] may play a role in the decreased calcium absorption, possibly because of decreased sensitivity of the 25 (OH)D-1α-hydroxylase to parathyroid hormone or impaired activity of the renal 25 (OH)D-1α-hydroxylase.

Genetic factors influence bone mass. Indeed, in identical twins, as much as 80 percent of age-specific variation in bone mass can be accounted for on a genetic basis.

Post Menopausal Osteoporosis

In her lifetime, a woman is overtly exposed to various hormones, which play a major role in determining her physical and mental well being. Every phase of a woman's life is marked by certain hormonal changes taking place within her body. Menopause is one such phase, which by far is the most crucial stage of woman's life.

Menopause, whether natural or artificial, marks the end of the childbearing years of a woman. Thanks to improved nutrition and disease control, today's woman spends one third of her life in the postmenopausal stage. It therefore becomes important to cease all pain & discomfort from the postmenopausal days and enable her to enjoy a healthy life beyond the fertile span.

In the postmenopausal phase, there is an increased risk of developing osteoporosis and cardiovascular complications. As per published data, over 61 million Indians have osteorporosis & 80% are women. It has been estimated that 35% of postmenopausal women in India are osteoporotic resulting in a high incidence of vertebral and non-vertebral (hip, wrist) fractures in elderly women.

Pathophysiology

Local production of cytokines appears to mediate the enhanced osteoclast-mediated bone resorption of estrogen deficiency. Peripheral blood monocytes from patients with osteoporosis secrete more interleukin (IL) 1, and the increased production of IL-1 in women with postmenopausal osteoporosis is suppressed by estrogen treatment. IL 1 and other cytokines such as tumor necrosis factor α (TNFα) stimulate production of IL-6 by osteoblasts and other mesenchymal cells. IL-6 is probably the most important cytokine in the recruitment of osteoclasts in the abnormal bone remodeling in postmenopausal osteoporosis. Although osteoporosis occurs with Cushing's syndrome, there is no established role for adrenal steroids in the osteoporosis associated with the menopause or advanced age.

Estrogen Treatment of Menopause

The main cause for postmenopausal osteoporosis is excessive bone resorption, which occurs due to estrogen deficiency. So the

condition was tried to be treated and /or prevented by supplementing postmenopausal women with estrogens. It was called hormone replacement therapy (HRT).

The rationale for use of estrogens in postmenopausal women is the belief that such therapy may relieve some of the complications of the postmenopausal state, including osteoporosis, and some manifestations of aging itself.

As is true for all estrogen therapy, the estrogen treatment of the menopause is actually a pharmacologic substitution of one or another estrogen analogue for estradiol rather than a physiologic replacement of the missing steroid. The estrogens available for replacement therapy include conjugated estrogens, estrogen substitutes (diethylstilbestrol), synthetic estrogen (ethinyl estradiol or derivatives), micronized estradiol, estrogen-containing vaginal creams, and estrogen-containing dermal patches.

Several lines of evidence indicate that routine estrogen therapy is beneficial in preventing the complications of menopausal osteoporosis, especially in high-risk women (i.e., thin white women). First, in women undergoing premature menopause, the incidence and complication rates of osteoporosis are increased, and long-term estrogen replacement appears to be beneficial. Second, estrogen therapy has short-term positive effects on calcium balance and long-term beneficial effects on

bone density. Third, in women given estrogen therapy, the incidence of fractures is decreased.

Side Effects of HRT

Of the potential side effects, the possibility of an increased risk of endometrial carcinoma is perhaps most worrisome. The relative risk of developing endometrial adenocarcinoma in estrogen users is between six and eight times the risk in nonusers. This risk increases with increasing duration and dosage of estrogen but is smaller in women given combination estrogen-progestogen therapy.

Recent large cohort studies suggest that estrogen and estrogen / progestin therapy may also be associated with an increased risk of breast cancer by a factor of 1.35 to 1.46 after five or more years of use.

Selective Estrogen Receptor Modulators

Specific estrogen receptor modulators (SERMs) are non-steroid molecules that maintain some of the agonist properties of estrogens on bone tissue and cardiovascular system, but not their stimulating effects on the gynecological sphere. **(Tremollieres F, Lopes P. Unite de menopause et maladies osseuses metaboliques, service d'endocrinologie, CHU Rangueil, Toulause)**

SERMs were formerly known as "antiestrogens" in reference to their primary inhibition of breast tumor growth. Hence, tamoxifen has been used for many years as adjuvant treatment of breast cancer.

However, its long-term use is limited by the risk of endometrial hyperplasia, which has led to the development of new molecules devoid of this side effect. Among these molecules, raloxifene, more specifically reserved for the prevention of osteoporosis in menopausal women, has been the subject of major preclinical and clinical developments. In the prevention of postmenopausal bone loss and vertebral fractures, the effects of raloxifene have been established in several randomized, double-blind studies against placebo, which were the basis of its current marketing authorization. Moreover, raloxifene has a favorable effect on lipid profile and, contrary to oral estrogens, does not increase the C-Reactive protein. Endometrial tolerance is good and it is associated with a significant reduction in the incidence of breast cancer in elderly osteoporotic women. Raloxifene's properties raise the question of its place, together with hormone replacement therapy (HRT), in the management of menopausal women. Its absence of efficacy in the control of the climacteric syndrome does not a priori make it a treatment choice at the beginning of postmenopausal phase. However, its effects in the prevention of vertebral fracture, its good gynecological tolerance and the fact that it is easy to administer, are arguments for its administration in the prevention of osteoporosis in 60 year-old women or in relay to HRT. Its safety on gynecological level privileges its use in all

women exhibiting benign breast or uterine pathologies at the origin of poor tolerance to HRT.

Comparison of Raloxifene and Hormone Replacement Therapy Adverse Events.

Raloxifene was compared with estrogen-progestin replacement therapy (HRT) in 3 clinical trials for prevention of osteoporosis. The table below shows adverse events occurring more frequently in one treatment group and at an incidence >/=2.0% in any group. Adverse events are shown without attribution of causality.

Adverse events reported in the clinical trials for osteoporosis prevention with RALOXIFENE (60 mg once daily) and continuous combined or cyclic estrogen plus progestin (HRT) at an incidence >/=2.0% in any treatment group.[a]

Adverse Events	RALOXIFENE (N=317) %	HRT-Continuous Combined (N=96) %	HRT-Cyclic (N=219) %
Urogenital			
Breast Pain	4.4	37.5	29.7
Vaginal Bleeding [b]	6.2	64.2	88.5
Digestive			
Flatulence	1.6	12.5	6.4
Cardiovascular			
Hot Flashes	28.7	3.1	5.9
Body as a Whole			
Infection	11.0	0	6.8
Abdominal Pain	6.6	10.4	18.7
Chest Pain	2.8	0	0.5

[a] These data are from both blinded and open-label studies.

[b] Treatment-emergent uterine-related adverse event, including only patients with an intact uterus: RALOXIFENE, n=290, HRT – Continuous Combined, n,=67, HRT-Cyclic, n=217.

Continuous Combined HRT = 0.625 conjugated estrogens plus 2.5 mg medroxyprogesterone acetate .
Cyclic HRT = 0.625mg conjugated estrogens for 28 days with concomitant 5mg medroxyprogesterone acetate or 0.15 mg norgestral on days I through 14 or 17 through 28.

Less side effect profile of SERM Raloxifene also offers an advantage for compliance over routine HRT.

A study was conducted by **Kayser J, Ettinger B, Pressman A (Menopause 2001 September-October, 8(5): 328-332)** with the objective to determine possible differences in continuation among postmenopausal women more than 60 years initiating treatment with the selective estrogen receptor modulator raloxifene, versus those initiating treatment with estrogen-containing regimens in postmenopausal women > 60 years of age .

At 24 months, the probabilities at discontinuing were 56% for women starting raloxifene compared to 72% for women starting estrogens. The likelihood of discontinuation was significantly less among women starting raloxifene than among those starting estrogen (hazard ratio = 0.75: 95% confidence interval = 0.64-0.88). Adjustments for age and prescriber specialty did not affect the risk.

We conclude that discontinuation of estrogen by women well beyond the age of menopause is high; more than two-thirds discontinue within 2 years of starting. Women starting therapy with raloxifene are 25% percent less likely to discontinue their medication than those starting estrogen, providing some promise that long-term benefits of raloxifene may be more easily achieved than those of estrogen.

Raloxifene-A Novel SERM

Raloxifene is a selective estrogen receptor modulator that partially mimics the effects of estrogens in bone and the cardiovascular system, while functioning as an antiestrogen in endometrial and breast tissue.

Raloxifene hydrochloride belongs to the benzothiophene class of compounds.

The chemical designation is methanone, [6-hydroxy-2-(4-hydroxyphenyl)benzo[b]thein-3-yl][-4[2-(1-piperidinyl)ethoxy]phenyl]-hydrochloride.Raloxifene hydrochloride (HCl) has the empirical formula C_{28} H_{27} NO_4 S . HCl, which corresponds to a molecular weight of 510.05. Raloxifene HCl is an off white to pale-yellow solid that is very slightly soluble in water.

Mechanism of Action :

The physiological effects of estrogens are mediated through their interaction with estrogen receptors, which are ligand- inducible nuclear transcription factors. .Binding of estrogen to its receptor facilitates the activation or repression of target genes through a series of molecular events including direct interaction of the estrogen – receptor complex with a DNA sequence, the estrogen response element (ERE), located upstream of target genes **(Macgregor JI, Jordan VC , Basic guide to the mechanisms of antiestrogen action , Pharmacol Rev 1998;50:151-96.)**

Although the precise mechanism of action of raloxifene remains to be elucidated , its tissue selective effects may be mediated by a number of mechanisms including differential interactions with estrogen receptors and cellular coactivators /respressors or at the DNA level. **(Khovidhunkit W, Shoback DM, Clinical effects of raloxifene hydrochloride in women. Ann intern Med 1999;130:431-9.)**

Indeed, recent work indicates that raloxifene may also influence gene transcription, via the intermediary of the estrogen receptor, by interacting with a DNA site distinct from the ERE. This molecular target has been termed the raloxifene response element (RRE). **(Yang NN, Venugopalan M, Hardikar S, et al. Identification of an estrogen response element activated by metabolities of 17-beta-estradiol and raloxifene . Science 1996, 273: 1222-5.)**

Transforming growth factor (TGF) -β3 is an important regulator of bone remodelling and its expression is up–regulated by raloxifene both in vitro and in vivo . In transfected human MG63 osteosarcoma cells, raloxifene stimulated a TGF-β3 responsive gene reporter system. Compared with control cells, an increase ($p<0.01$) in TGFβ3 activation was observed at concentrations of raloxifene as low as 1 nmol/L. In contrast, 100-fold higher concentrations of 17β - estradiol were required to produce a significant increase .Raloxifene -and 17β - estradiol -

induced up- regulation of TGF-β3 activity was dependent on the presence of a functional estrogen receptor. **(Yang NN, Bryant, HU, Hardikar S, et al. Estrogen and raloxifene stimulate transforming growth factor- beta 3 gene expression in rat bone: a potential mechanism for estrogen - or raloxifene- mediated bone maintenance . Endocrinology 1996; 137:2075-84.)**

Clinical Effects

1. Effects on Bone:

Bone remodeling

Raloxifene has in vitro effects on bone cell homeostasis and favorably alters BMD, bone quality and strength in animal models. Raloxifene also has positive effects on biochemical markers of bone turnover, histomorphometric parameters and calcium balance in postmenopausal women.**(Krane SM, Holick MF. Metabolic bone disease. In :Fauci AS, Braunwald E, Isselbacher KJ et al , editors . Harrisons principles of internal medicine. 14th ed . vol 2. New York: McGraw-Hill,1999;2247-53.)**

Bone cell homeostasis

Raloxifene modulates bone cell homeostasis in vitro through actions on the proliferation and activity of osteoclasts and osteoblasts. In murine neonatal bone marrow cultures, raloxifene 1 pmol/L to 1 μmol/L

concentration-dependently inhibited vitamin D3-induced osteoclast differentiation. **(Migliaccio S, Teti A, Taranta A, et al. Raloxifene modulates osteoclastogenesis and bone resorption in vitro (abstract). Abstracts of the 3rd international Symposium on Women's Health and Menopause; 1998 Jun 13-16, Florence: 19.)**

A concentration-dependent increase in the proliferation of osteoblasts from neonatal murine calvaria was reported after exposure to raloxifene..**(Migliaccio S, Taranta A, Teti A, et al. Raloxifene directly modulates bone cell activity in vitro (abstract). Bone 1998; 23 Suppl.: 608).** Expression of the bone matrix proteins alkaline phosphatase, osteonectin and osteocalcin was increased. Additionally, raloxifene stimulated collagen expression by mature osteoblasts in a mineralized matrix. Expression of interleukin (IL)-6 was markedly inhibited by raloxifene. The effects of raloxifene on bone matrix proteins were similar to those of estrogen. **(Lengner C, Green J, Bodine PVN, et al. Selective modulation of osteoblast growth and differentiation parameters by antiestrogens(abstract). Bone 1998;23(5) Suppl.: S206)**

In postmenopausal women with osteoporosis, raloxifene reduced the risk of vertebral fractures. Raloxifene also increased bone mineral density (BMD) of the spine, hip and total body. Similarly, in early postmenopausal women without osteoporosis (women with normal or

low BMD without fracture), Raloxifene increased spine, hip and total body BMD relative to calcium alone at 24 months. The effect on hip bone mass was similar to that for the spine.

Raloxifene (60mg once daily) related increases in BMD for the osetoporosis treatment study expressed as mean percentage versus placebo.[a b]

	Time		
Site	**12 Months** **%**	**24 Months** **%**	**36 Months** **%**
Lumbar Spine	2.0	2.6	2.6
Femoral Neck	1.3	1.9	2.1
Ultradistal Radius	ND	2.2	ND
Distal Radius	ND	0.9	ND
Total Body	ND	1.1	ND
Note: All BMD increase were significant ($p<0.001$)			
[a] Intent-to-treat analysis; last observation carried forward			
[b] All patients received calcium and vitamin D.			
ND = not done (total body and radius BMD were measured only at 24 months)			

The effects of raloxifene on fracture incidence and BMD in postmenopausal women with osteoporosis were examined at 3 years in a large randomized placebo-controlled, double-blind multinational osteoporosis treatment trial. All vertebral fractures were diagnosed radio

graphically; some of these fractures also were associated with symptoms (i.e., clinical fractures). The study populations consisted of 7705 postmenopausal women with osteoporosis as defined by : a) low BMD (vertebral or hip bone mineral density at least 2.5 standard deviations below the mean value for healthy young women) without baseline vertebral fractures, or b) one or more baseline vertebral fractures. Women enrolled in this study had a median age of 67 years (range 31 to 80) and a median time since menopause of 19 years.

Raloxifene, 60 mg administered once daily, increased spine and hip BMD by 2-3%. Raloxifene decreased the incidence of the first vertebral fracture from 4.3% for placebo to 1.9% for raloxifene (relative risk reduction=55%) and subsequent vertebral fractures from 20.2% for placebo to 14.1% for raloxifene (relative risk reduction = 30%) All women in the study received calcium 500mg/day and vitamin D(400-600IU/day). Raloxifene reduced the incidence of vertebral fractures whether or not patients had a vertebral fracture upon study entry. The decrease in incidence of vertebral fracture was greater than could be accounted for by increase in BMD alone.

Effect of Raloxifene on Risk of Vertebral Fractures

	Number of Patients		Absolute Risk Reduction	Relative Risk Reduction (95% CI)
	Raloxifene	Placebo		
Fractures diagnosed radiographically				
Patients with no baseline fracture [a]	n = 1401	n = 1457		
Number (%) of patients with >/=1 new vertebral fracture	27 (1.9%)	62 (4.3%)	2.4%	55% (29%, 71%)
Patients with >/=1 baseline fracture [a]	n=858	n=835		
Number (%) of patients with >/=1 new vertebral fracture	121 (14.1%)	169 (20.2%)	6.1%	30% (14%, 44%)
Symptomatic vertebral fractures				
All randomized patients	n=2557	n=2576		
Number (%) of patients with >/=1 new clinical (painful) vertebral fracture	47(1.8%)	81(3.1%)	1.3%	41% (17%,59%)

The mean percentage change in BMD from baseline for raloxifene was statistically significantly greater than for placebo at each skeletal site.

Discontinuation from the study was required when excessive bone loss or multiple incident vertebral fractures occurred. Such discontinuation was statistically significantly more frequent in the placebo group (3.7%) than in the raloxifene group (1.1%)

Prevention of Osteoporosis

The effects of raloxifene on bone mineral density (BMD) in postmenopausal women were examined in three randomized, placebo-controlled, double-blind osteoporosis prevention trials: (1) a North

American trial enrolled 544 women; (2) a European trial, 601 women; and (3) an international trial, 619 women who had undergone hysterectomy. In these trials, all women received calcium supplementation (400 to 600 mg/day). Women enrolled in these studies had a median age of 54 years and a median time since menopause of 5 years (less than 1 year up to 15 years post menopause). The majority of the women were Caucasian (93.5%). Women were included if they had spine bone mineral density between 2.5 standard deviations below and 2 standard deviations above the mean value for healthy young women. The mean T scores (number of standard deviations above or below the mean) for the 3 studies ranged from -1.01 to -0.74 for spine BMD and included women both with normal and low BMD. Raloxifene, 60 mg administered once daily, produced increases in bone mass versus calcium supplementation alone, as reflected by dual-energy x-ray absorptiometric (DXA) measurements of hip, spine and total body BMD . Compared with placebo, the increases in BMD for each of the 3 studies were statistically significant at 12 months and were maintained at 24 months .The placebo groups lost approximately 1% of BMD over 24 months.

Raloxifene (60 mg once daily) related increases in BMD for the three osteoporosis prevention studies expressed as mean percentage increase versus placebo [a] at 24 months [b]			
	Study		
Site	NA %	EU %	INT[c] %
Total Hip	2.0	2.4	1.3
Femoral Neck	2.1	2.5	1.6
Trochanter	2.2	2.7	1.3
Intertrochanter	2.3	2.4	1.3
Lumbar Spine	2.0	2.4	1.8
Abbreviations : NA = North American, EU = European, INT = International			
Note: all BMD increases were significant (p</=0.001)			
[a] All patients received calcium			
[b] Intent-to-treat analysis; last observation carried forward			
[c] All women in the study had previously undergone hysterectomy.			

Raloxifene also increased BMD compared with placebo in the total body by 1.3% to 2.0% and in Ward's Triangle (hip) by 3.1% to 4.0% .

Effect on Bone Metabolism

In a 31-week open-label radiocalcium kinetics , 33 early postmenopausal women randomized to treatment with once-daily raloxifene 60 mg, cyclic estrogen /progestin (0.625mg conjugated estrogens daily with 5mg medroxyprogesterone acetate daily for the first two weeks of each months [HRT]), or no treatment. Treatment with either raloxifene or HRT was associated with reduced bone resorption

and a positive shift in calcium balance (-82mg Ca/day and +60mg Ca/day, respectively for raloxifene and -162 mg Ca/day and+91 mg Ca/day, respectively for HRT).

In both the osteoporosis treatment and prevention trials, raloxifene therapy resulted in consistent, statistically significant suppression of bone resorption and bone formation, as reflected by changes in serum and urine markers of bone turnover (e. g., bone-specific alkaline phosphatase, osteocalcin, and collagen breakdown products). The suppression of bone turnover markers was evident by 3 months and persisted throughout the 36-month and 24-month observation periods.

A study conducted by **Prashanti Edwards et al 2002** found no correlation between serum calcium and age or BMD.

The mean serum calcium values in perimenopausal (n=42), postmenopausal (n=45), posthysterectomy (n=33) was 9.320$\pm$ 0.603 mg/day, 9.237$\pm$0.552, 9.015$\pm$ 0.613 respectively.

The mean value (n=120) was 9.800$\pm$ 0.595. There was no significant correlation with raloxifene therapy or changes in BMD with raloxifene therapy However ,there was a significant decline in bone turnover rates when treated with raloxifene for 6 months or more. The markers of bone turnover like serum osteocalcin ,, serum bone specifie alkaline posphatase,urinary CTx/Cr (c- telopeptide normalized to creatinine)and urinary NTx/Cr (N telopeptide normalized to creatinine)

values declined significantly with raloxifene 60mg/day. Ultradistal BMD increased 0.2% with raloxifene 60mg/day compared to controls where it decreased by 2.7% over baseline. This effect was statistically significant.

Effects of oral once daily Raloxifene (RLX) on markers of bone turnover in randomized double-blind studies of > 6 months' duration involving postmenopausal women or patients with Osteoporosis.[a,4]

Reference	Study duration (mo)	Mean age (y)	Mean duration of menopause (y)	Treatment[b] (mg/day) [no. patients randomized]	Markers of bone turnover (% change from baseline at end-point)			
					Serum OCN	Serum BSAP	Urinary CTx/Cr	Urinary NTx/Cr
Postmenopausal Women								
Delmas et al.	36[d]	55	4-5	RLX 30[152]	-21.8*	-14.2*		
Bjarnason et al [c].				RLX 60[152]	-26.1*	-18.8*		
				RLX 150[157]	-29.0*	-19.9*		
				PL [150]	-10.9	-12.9		
Gunness et al [c]	6	64.4	18.2	RLX 60[25]	-11 [#]	-16 [#]		-25
				CEE 0.625[26]	-26	-40		-48
Postmenopausal patients with osteoporosis								
Ettinger et al.	36	65-69	17-21	RLX 60[2259]	-26.3***		-34.0***	
				RLX 120[2277]	-31.1***		-31.5***	
				PL [2292]	-8.6		-8.1	
Johnell et al. [c]	12	NR	≥2	RLX 60 [82]	-25.5 *[$]	-24.4 *[$]	-31.2*[$]	-17.8*[$]
				ALN 10[83]	-38.8*	-45.3*	-49.5*	-40.9*
				RLX 60+ ALN 10 [84]	-48.8*	-48.4*	-69.3*	-69.3*
				PL [81]	5.0	-10.6	4.4	26.9
Lufkin et al.	12	67.2-69.9	22.0-23.5	RLX 60[48]	-33.1***	-36.0**	-35.9**	
				RLX 120[47]	-29.4**	-30.0*	-41.8*	
				CON[48]	-12.4	-21.1	-11.0	
Meunier et al.	24	59.2-60.2	11.7-13.2	RLX 60[45]	-28.4***	-13.7**		
				RLX 150 [42]	-25.2***	-15.8***		
				PL [42]	-0.7	4.1		

a) Ostoporosis was defined as BMD values of less than – 2.5 SD relative to normal premenopausal women and / or ≥ 1 moderate or severe vertebral fracture, a femoral neck BMD > 2 SD below premenopausal peak BMD, a BMD ≤ 10th percentile of normal premenopausal women plus ≥ 1 nontraumatic vertebral fracture or a BMD of < -1.0 SD relative to the age-adjusted normal population.

b) Participants received supplements of Vitamin D_3 300 to 800 IU/day and /or calcium up to 1000 mg/day.

c) Abstract

d) 36-month data reported by Bjarnason et al.

Control treatment was supplementation with calcium 750 mg/day and vitamin D3 800 IU/day (also received by Raloxifene-treated patients). ALN=alendronate, BSAP=bone-specific alkaline phosphatase, CEE=conjugated equine estrogens; CON = control; CTxCr=C-telopeptiede normalized to creatinine, NR = not reported, NTxCr = N-telopeptide normalized to creatinine, OCN= osteocalcin; PL=Placebo; *p<0.05, **p<b.01. ***p≤0.001 vs PL or CON [#]p<0.05 vs CEE [$]p<0.05 vs RLX + ALN

Bone Histomorphometry

In the treatment study, bone biopsies for qualitative and quantitative histomorphometry were obtained at baseline and after 2 years of treatment. There were 56 paired biopsies evaluable for all indices. In raloxifene-treated patients, there were statistically significant decreases in bone formation rate per tissue volume, consistent with a reduction in bone turnover. Normal bone quality was maintained; specifically, there was no evidence of osteomalacia, marrow fibrosis, cellular toxicity or woven bone after 2 years of treatment.

2. Cardiovascular Benefits

In postmenopausal women, raloxifene favorably alters several markers of cardiovascular risk including lipid parameters.

Raloxifene 60 mg/day significantly reduced serum levels of total and low density lipoprotein cholesterol from baseline, compared with placebo. High density lipoprotein cholesterol and triglyceride levels were unaffected.

Significant reductions in serum levels of homocysteine and tumor necrosis factor-α were reported during raloxifene treatment and these effects were similar to those of estrogens. **(D.Clemett, C.M.Spencer – Drugs 2000 Aug 60(2) Pg 379-411)**

Effects on Other Markers of Cardiovascular Risk

Raloxifene 60 mg/day had a number of effects on other marks of cardiovascular risk in postmenopausal women:

- Serum fibrinogen level were reduced by 12% from baseline, compared with 2% with placebo treatment over 24 months
- A 9% reduction in serum apoliporotein B levels was reported after 24 months' treatment whereas levels were unchanged with placebo.
- Serum homocysteine levels were reduced by 8% after 6 months' treatment whereas levels were unchanged with placebo
- 6 months' treatment reduced serum tumor necrosis factorα (TNFα) levels by 13.5% compared with an increase of 106% among placebo recipients.

3. Antiatherogenic Effects

The oxidation of LDL-cholesterol particles has been reported to play an important role in the pathogenesis of atherosclerosis. In an in vitro study, raloxifene had antioxidant effects on human LDL-cholesterol particles. Copper sulphate-mediated LDL oxidation was dose-dependently inhibited in the presence of raloxifene. The antioxidant effect of raloxifene was greater than that of 17 β estradiol.

4. Effects on Breast Tissue

The latest report from the **MORE Study** indicates that among postmenopausal woman with osteoporosis, the risk of invasive breast cancer was decreased by 76% during 3 years of treatment with Raloxifene, when compared to placebo. Raloxifene reduced the risk of invasive estrogen receptor-positive breast cancer by 90% without influencing receptor-negative tumors. Several studies have indicated that Raloxifene has in vitro antiproliferative effects on human breast cancer cells and inhibits mammary carcinogenesis in animal models of breast cancer.

A 76% reduction in risk of invasive breast cancer was reported for the pooled Raloxifene group, and the reduction in risk was similar between 60 and 120 mg/day dosage groups. Of the 35 cases of invasive cancer for which estrogen receptor status was known, 24 were estrogen-receptor positive and 11 were negative. Raloxifene reduced the risk of estrogen-receptor positive breast cancer but did not affect estrogen receptor negative cancer risk.

5. Effects on Endometrial Tissue

One particular advantage of raloxifene over hormone replacement therapy is its apparent lack of proliferative effects on endometrial tissue. Two six-months studies involving a total of 969 postmenopausal women showed that endometrial thickness did not differ between women

receiving raloxifene (30 to 150 mg per day) and those receiving placebo. **(J.A. Scott ,CC. Da Camara, J.E. Early , Am Fam Physician 1999;60:pg 1131-1139.).**

Pharmacokinetics

The disposition of raloxifene has been evaluated in more than 300 postmenopausal women in selected raloxifene osteoporosis treatment and prevention clinical trials using a population approach. Pharmacokinetic data were also obtained in conventional pharmacology studies in 292 postmenopausal women. Raloxifene exhibits high within-subject variability (approximately 30% coefficient of variation) of most pharmacokinetic parameters.

Summary of raloxifene pharmacokinetic parameters in the healthy postmenopausal woman

	C_{max}[a] (ng/ mL)/ (mg/ kg)	T ½ (hr)	$AUC_{0(infinity)}$[a] (ng-hr/ mL)/ (mg/kg)	CL/F (L/kg-hr)	V/F (L/kg)
Single Dose					
Mean	0.50	27.7	27.2	44.1	2348
CV(%)	52	10.7 to 273[b]	44	46	52
Multiple Dose					
Mean	1.36	32.5	24.2	47.4	2853
CV(%)	37	15.8 to 86.6[b]	36	41	56
Abbreviations: C_{max} = maximum plasma concentration, t ½ = half-life, AUC = area under the curve, CL = clearance, V = volume of distribution, F = bioavailability, CV= coefficient of variation.					
[a] Data normalized for dose in mg and body weight in kg.					
[b] Range of observed half-life					

Special Populations

Geriatric - No differences in raloxifene pharmacokinetics were detected with regard to age (range 42 to 84 years).

Pediatric - The pharmacokinetics of raloxifene have not been evaluated in a pediatric population.

Gender - Total extent of exposure and oral clearance, normalized for lean body weight, are not significantly different between age-matched female and male volunteers.

Race - There are no discernible differences in raloxifene plasma concentrations among various racial groups; however, the influence of race cannot be conclusively determined.

Renal Insufficiency – In the osteoporosis treatment and prevention trials, raloxifene and metabolite concentrations in women with estimated creatinine clearance as low as 21 ml/min are similar to women with normal creatinine clearance.

Hepatic Dysfunction –Safety and efficacy have not been evaluated further in patients with hepatic insufficiency.

Contraindications

Raloxifene is contraindicated in lactating women or women who are or may become pregnant. Raloxifene may cause fetal harm when administered to a pregnant woman. In rabbit studies, abortion and a low rate of fetal heart anomalies (ventricular septal defects) occurred in

rabbits at doses>/=0.1 mg/kg (>/=0.04 times the human dose based on surface area, mg/ m^2), and hydrocephaly was observed in fetuses at doses>/=10 mg/kg(>/=4 times the human doses based on surface area mg/m^2).

Raloxifene is contraindicated in women with active or past history of venous thromboembolic events, including deep vein thrombosis, pulmonary embolism, and retinal vein thrombosis.

Raloxifene is contraindicated in women known to be hypersensitive to raloxifene or other constituents of the tablets.

Warnings

Venous Thromboembolism – In clinical trials, raloxifene - treated women had an increased risk of venous thromboembolism (deep vein thrombosis and pulmonary embolism). Other venous thromboembolic events could also occur. A less serious event, superficial thrombophlebitis, also has been reported more frequently with raloxifene. The greatest risk for deep vein thrombosis and pulmonary embolism occurs during the first 4 months of treatment. Because immobilization increases the risk for venous thromboembolic events independent of therapy, raloxifene should be discontinued at least 72 hours prior to and during prolonged immobilization (e. g., post-surgical recovery, prolonged bed rest), and patients should be advised to move about periodically during prolonged travel.

Premenopausal Use – There is no indication for premenopausal use of raloxifene. Safety of raloxifene in premenopausal woman has not been established and its use not recommended .

Hepatic Dysfunction – Safety and efficacy have not been evaluated further in patients with severe hepatic insufficiency.

Precautions

For safe and effective use of raloxifene, the physician should inform patients about the following:

Patient Immobilization – Raloxifene should be discontinued at least 72 hours prior to and during prolonged immobilization (e. g., post-surgical recovery, prolonged bed rest), and patients should be advised to avoid prolonged restriction of movement during travel because of the increased risk of venous thromboembolic events.

Hot Flashes or Flushes -- Raloxifene may increase the incidence of hot flashes and is not effective in reducing hot flashes or flushes associated with estrogen deficiency. In some asymptomatic patients, hot flashes may occur upon beginning raloxifene therapy.

Other Osteoporosis Treatment and Prevention Measures – Patients should be instructed to take supplemental calcium and/or vitamin D, if daily dietary intake is inadequate. Weight-bearing exercise should be considered along with the modification of certain behavioral factors, such

as cigarette smoking, and and/or alcohol consumption, if these factors exist.

Side Effects

The majority of adverse events occurring during clinical trials were mild and generally did not require discontinuation of therapy.

Vasomotor Effects

Three-year data from the large (n = 7705) osteoporosis treatment study indicated that the incidence of hot flushes was higher ($p < 0.001$ Vs placebo) during raloxifene 60 or 120 mg/day treatment (9.7 and 11.6%, respectively) than with placebo (6.4%). **(Ettinger B, Black DM, Mitlak BH, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results form a 3- year randomized clinical trial. JAMA1999; 282: 637- 45.)** Most episodes of hot flushes were mild or moderate in intensity (22.5 and 16.4% incidence in raloxifene and placebo groups, respectively) and the frequency of severe hot flushes did not differ between groups (2.1 Vs 1.9%). Although the incidence of treatment-emergent hot flushes was greater with raloxifene than in the placebo group in the first 6 months (21.0 Vs 14.4%, $p < 0.001$), there was no significant difference between groups beyond 6 months of treatment (7.6 Vs 6.9%).**(Davies GC , Huster WJ, Lu Y. Adverse events reported by postmenoenopausal women in controlled trials with raloxifene .**

Obstet Gynecol 1999; 93: 558- 65.) The occurrence of other vasomotor symptoms such as daytime and night sweats was not significantly different between raloxifene and its comparators in placebo- (3.1 Vs 1.7% in raloxifene and comparator groups, respectively). **(Cohen FJ, Lu Y. Characterization of hot flashes reported by healthy postmenopausal women receiving raloxifene or placebo during osteoporosis prevention trials. Maturtas 2000; 34: 65-73)**

Leg Cramps

In 6- to 30- month placebo-controlled studies, the incidence of leg cramps was higher ($p < 0.05$) in the pooled raloxifene group than among those receiving placebo (5.5 Vs 1.9%).

A small, but significantly greater incidence of peripheral oedema was also reported in patients receiving raloxifene compared with placebo in this study (5.2, 6.5 and 4.4% in raloxifene 60 or 120 mg/day and placebo groups, $p < 0.01$ for combined raloxifene dosages Vs placebo).

Urogenital Events

The incidence of vaginal bleeding was low and similar to that with placebo during treatment with raloxifene 60 mg/day.

Urinary incontinence occurred more frequently in patients receiving unopposed estrogens than those treated with raloxifene (4.3 Vs 0%, $p < 0.05$), although the incidence of this event did not differ between other treatment groups.

Breast Pain

Breast pain was uncommon during placebo-controlled studies and occurred in similar percentages of individuals in raloxifene 60 mg/day and placebo groups .

In HRT comparative studies, breast pain was reported to be moderate or severe in 7.6% of those receiving HRT but was not reported at this severity in the raloxifene group ($p < 0.001$).

Serious Adverse Events

The only serious adverse events considered related to raloxifene treatment were venous thromboembolic episodes, including deep venous thrombosis and pulmonary embolism. At 40 months, the risk of venous thromboembolic events was elevated in patients treated with raloxifene (1% in each of 60 and 120 mg/day groups) compared with those receiving placebo (0.3%;)

MATERIAL
&
METHODS

The patients selected for our study were taken from the postmenopausal women attending the OPD and inpatient department of Obstetrics and Gynaecology, M.L.B. Medical College , Jhansi .

A detailed history of all patients was taken including name, age, parity, socioeconomic status, occupation, residence. Other parameters included:- age of menopause, duration of menopause, type of menopause- whether natural or artificial, previous menstrual history ,obstetrical history , personal history(ie., history of smoking, alcohol intake), drug history, past medical & surgical history (history of tuberculosis, hypertension, diabetes mellitus, ischemic heart disease and history of any surgery).

All women selected for study were subjected to complete general examination, including general condition, pulse, blood pressure, body temperature, respiratory rate, pallor, cyanosis, jaundice, edema, lymphadenopathy.

Our study is an effort to use the drug in Indian women for both treatment & prevention of postmenopausal osteoporosis and compare its benefits with the international trials. We have taken bone mineral density & serum calcium as markers for the

therapeutic effect of raloxifene and compared it with no hormonal therapy in our study.

The patient's bone mineral density (BMD) and serum calcium values were noted at the beginning of the study. Based on bone mineral density values, the patients were initially classified as:-

Osteoporotic - **BMD < 0.425 gm/cm^2**

Osteopenic - **BMD in-between 0.425gm/cm^2 and 0.510gm/cm^2**

Normal - **BMD >0.510gm/cm^2**

100 subjects, who had attained menopause, either naturally or surgically, were taken into the study. 50 of these with normal bone mineral density were included into the "Prevention Trial Group" and 50 postmenopausal women with less than normal bone mineral density were included in the "Treatment Trial Group".

I. Prevention Trial Group:-

This group comprised 50 subjects with normal bone mineral density . It was further subdivided into:-

(A). Study group – It consisted of 25 postmenopausal women who were given raloxifene 60mg/day and calcium 500mg/day for a period of one year.

(B). Control group- It included 25 subjects who were given only calcium 500mg/day for a year.

II. Treatment Trial Group:-

This group was composed of 50 postmenopausal women with less than normal bone mineral density. It was further subdivided into:-

(A) Study group: - It consisted of 25 postmenopausal women who were prescribed raloxifene 60mg/day along with calcium 500mg/day for a period of one year.

(B) Control group: - It included 25 postmenopausal women who were given only calcium 500mg/day for one year.

The patient's bone mineral density was measured by Pronosco X-posure System (Dual Energy X- Ray Absorptiometry). It is a new technology that uses standard X-ray images of the hand and forearm. This new technique is based on radiogrammetry and offers automated detection of the Regions Of Interest (ROI's) followed by computerized image analysis. With the addition of digital scanning, the X-ray image is analyzed in detail. It is highly accurate, quick, precise and a low-cost tool for estimation of bone mineral density and also does not require special training of operators. The patient only needed to be present during the X-ray exposure.

After one year of therapy, the patients were evaluated for bone mineral density & serum calcium values.

The patients were also interviewed regularly for any side effects during the period of trial.

Exclusion Criteria:-

The following patients were excluded from the study:-

1. Those with known hypersensitivity to the drug.
2. Those with a past history of venous thromboembolic events including deep vein thrombosis, pulmonary embolism and retinal vein thrombosis.
3. Those with hepatic impairment including cholestasis/ jaundice.
4. Patients with unexplained vaginal bleeding.

OBSERVATIONS

TREATMENT STUDY

1. AGE CHARACTERISTICS OF SUBJECTS UNDER STUDY :

Age Group (years)	Cases		Controls		Total	
	n=25	%	N=25	%	N=50	%
31-40	0	0	0	0	0	0
41-50	3	12	3	12	6	12
51-60	14	56	12	48	26	52
61-70	8	32	10	40	18	36
Mean age	**57.5 ± 5.27 years**		**58.3 ± 5.45 years**			

2. DISTRIBUTION ACCORDING TO AGE OF ONSET OF MENOPAUSE :

Age Group (years)	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
31-40	1	4	0	0	1	2
41-50	10	40	13	52	23	46
51-60	14	56	12	48	26	52
61-70	0	0	0	0	0	0
Mean age	**50.7 ±4.47 years**		**50.3 ± 4.47 years**			

TREATMENT STUDY

AGE CHARACTERISTICS OF SUBJECTS UNDER STUDY

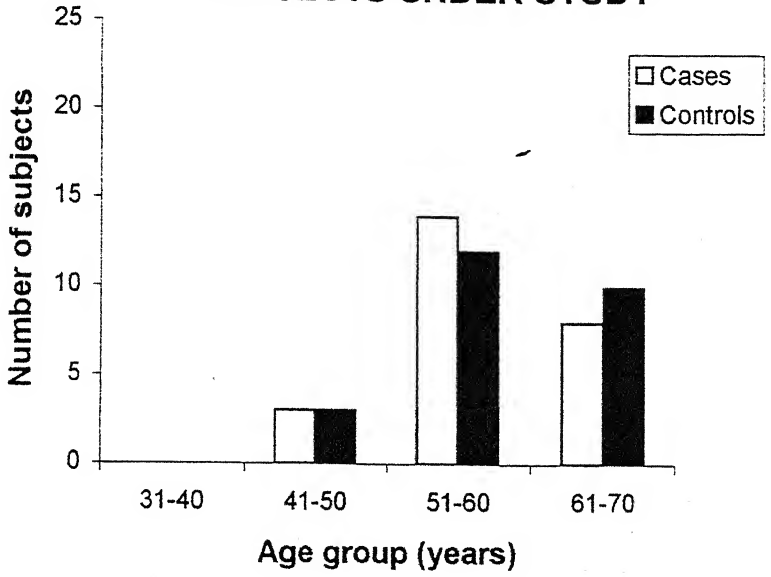

CHART NO. 1

DISTRIBUTION ACCORDING TO AGE OF ONSET OF MENOPAUSE

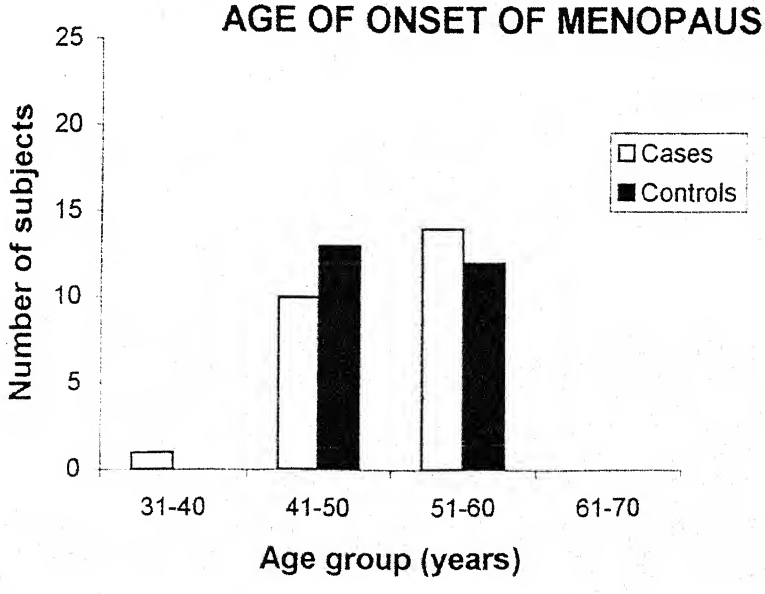

CHART NO.2

3. DISTRIBUTION ACCORDING TO DURATION OF MENOPAUSE :

Duration (years)	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
<1	0	0	0	0	0	0
1-5	3	12	3	12	6	12
6-10	16	64	14	56	30	60
11-15	6	24	8	32	14	28
Mean Duration	**8.48 ± 2.13 years**		**8.84 ± 1.99 years**			

4. DISTRIBUTION ACCORDING TO PARITY :

Parity	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
P1	0	0	0	0	0	0
P2	3	12	4	16	7	14
P3	10	40	12	48	22	44
P4	12	48	9	36	21	42
Mean parity	**3.36 ± 0.56**		**3.2 ± 0.52**			

DISTRIBUTION ACCORDING TO DURATION OF MENOPAUSE

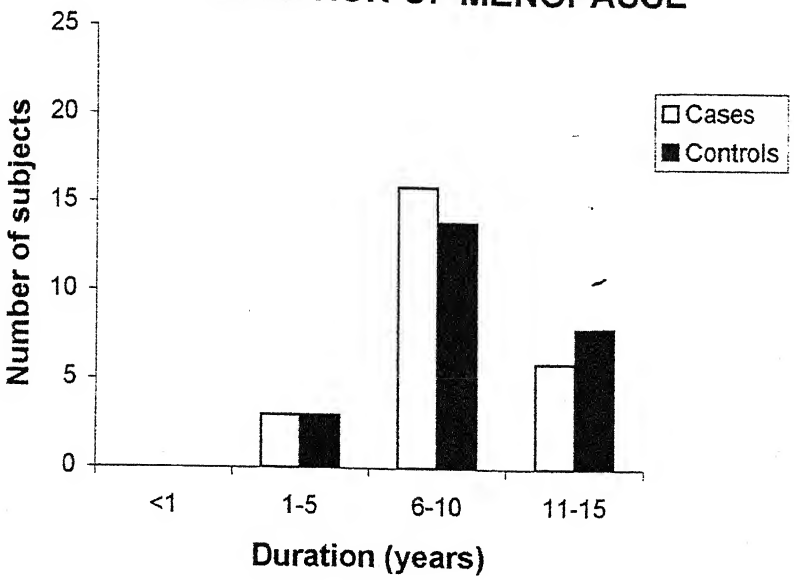

CHART NO.3

DISTRIBUTION ACCORDING TO PARITY

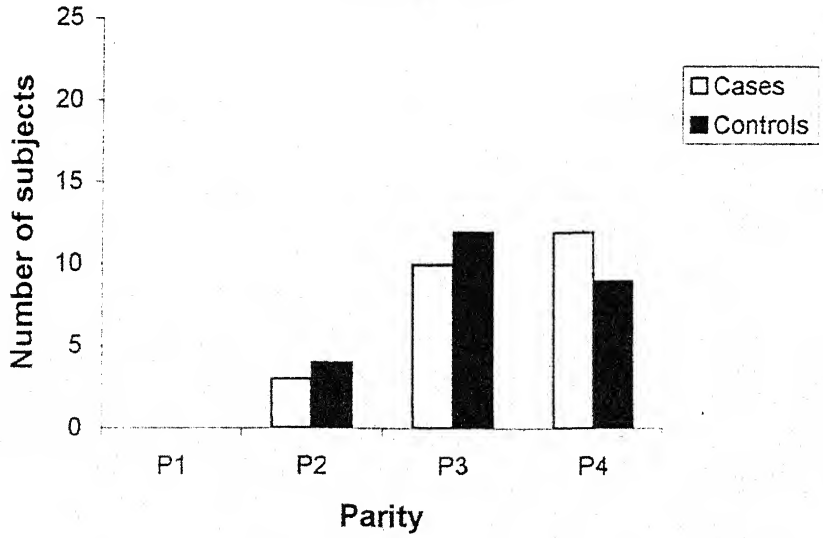

CHART NO.4

5. DISTRIBUTION ACCORDING TO TYPE OF MENOPAUSE :

Type	Cases		Controls	
	n=25	%	n=25	%
Natural	14	**56**	11	**44**
Surgical	11	**44**	14	**56**

6. DISTRIBUTION ACCORDING TO SOCIOECONOMIC STATUS :

Socioeconomic Status	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
Upper	0	0	0	0	0	0
Middle Upper	6	24	5	20	11	22
Middle Lower	**11**	**44**	**12**	**48**	**23**	**46**
Lower	8	32	8	32	16	32

DISTRIBUTION ACCORDING TO TYPE OF MENOPAUSE

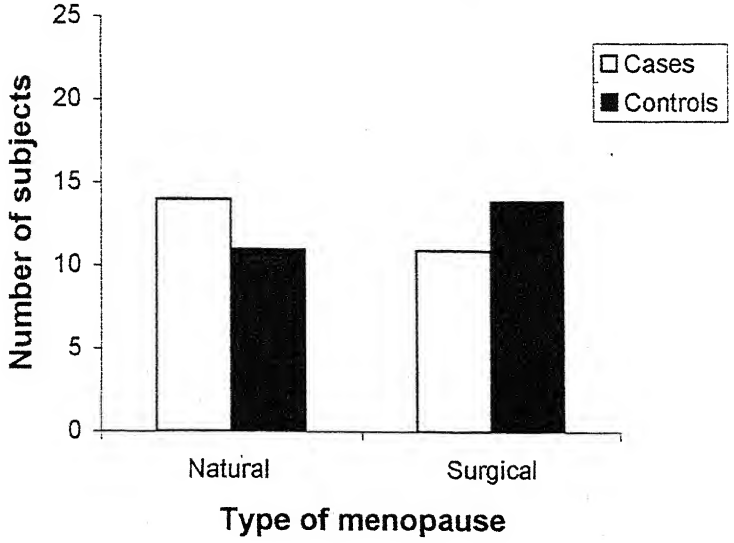

CHART NO.5

DISTRIBUTION ACCORDING TO SOCIOECONOMIC STATUS

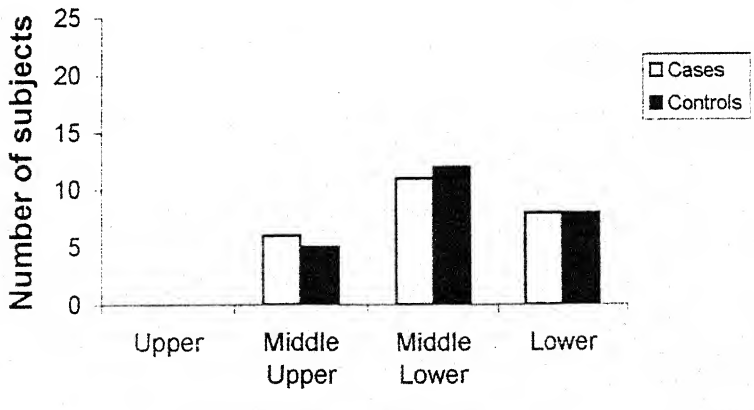

CHART NO.6

7. DISTRIBUTION OF CHANGE IN MEAN BMD :

	Cases	Controls
Mean BMD at start of study	0.431	0.434
Change in BMD after One Year	(+) 0.0074	(-) 0.0035
Percent Change	**(+) 1.71 %**	**(-) 0.86 %**

8. DISTRIBUTION OF PERCENTAGE CHANGE IN BMD :

Percentage BMD Change	Cases		Control	
	n=25	% (+)	n=25	% (-)
0	5	**20**	10	**40**
≤1%	1	**4**	3	**12**
1.1-2%	8	**32**	11	**44**
>2%	11	**44**	1	**4**

9. DISTRIBUTION OF MEAN BMD IN REFERENCE TO AGE :

	Cases	Controls
Age Group (years)	Mean BMD (at start of study)	Mean BMD (at start of study)
31-40	0	0
41-50	0.462	0.494
51-60	0.442	0.442
61-70	0.401	0.405

10. DISTRIBUTION OF MEAN BMD CHANGE IN STUDY POPULATION:

	Cases			Controls		
Age Group (years)	Mean BMD (at start of study)	Mean BMD (after one year)	**% Mean Change (+)**	Mean BMD (at start of study)	Mean BMD (after one year)	**% Mean Change (-)**
31-40	0	0	**0%**	0	0	**0%**
41-50	0.462	0.462	**0%**	0.494	0.493	**0.20%**
51-60	0.442	0.451	**2.04%**	0.442	0.438	**0.90%**
61-70	0.401	0.408	**1.75%**	0.405	0.401	**0.98%**

DISTRIBUTION OF MEAN BMD IN REFERENCE TO AGE

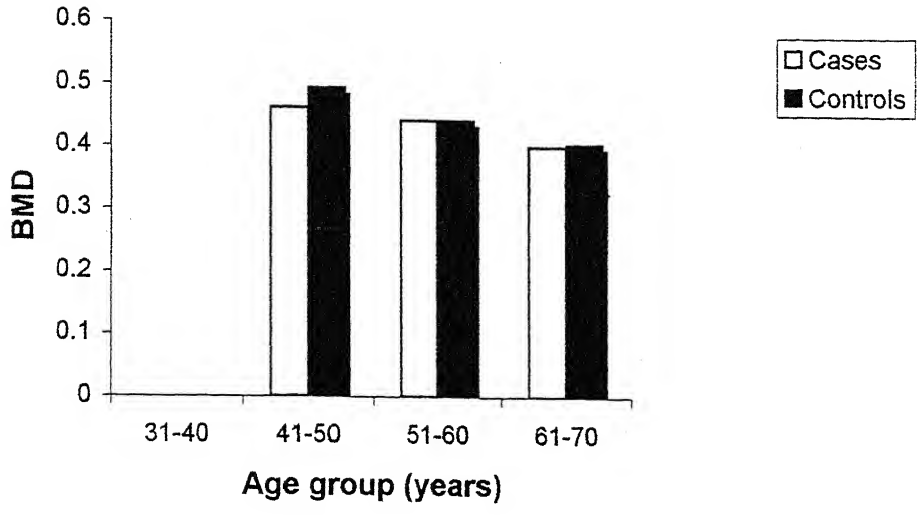

CHART NO.9

DISTRIBUTION OF MEAN BMD CHANGE IN STUDY POPULATION

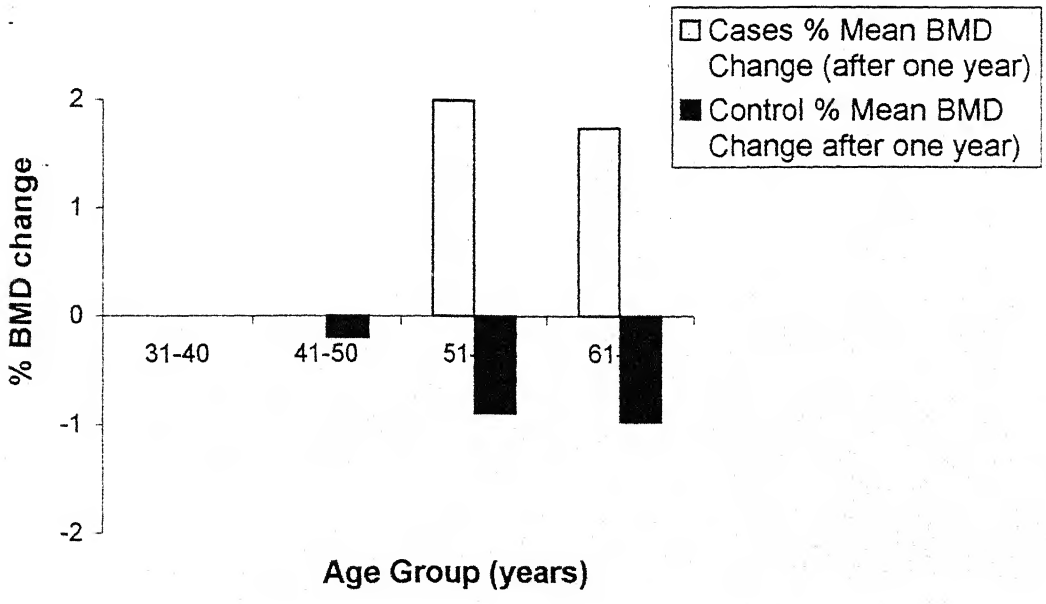

CHART NO.10

11. CHANGE IN MEAN SERUM CALCIUM VALUES :

	Cases	Controls
Mean serum calcium levels (at start of study)	9.43	9.43
Mean serum calcium levels (after one year)	9.43	9.51
Change in mean serum calcium levels	0	0.08
Percent change	**0**	**0**

CHANGE IN MEAN SERUM CALCIUM LEVEL

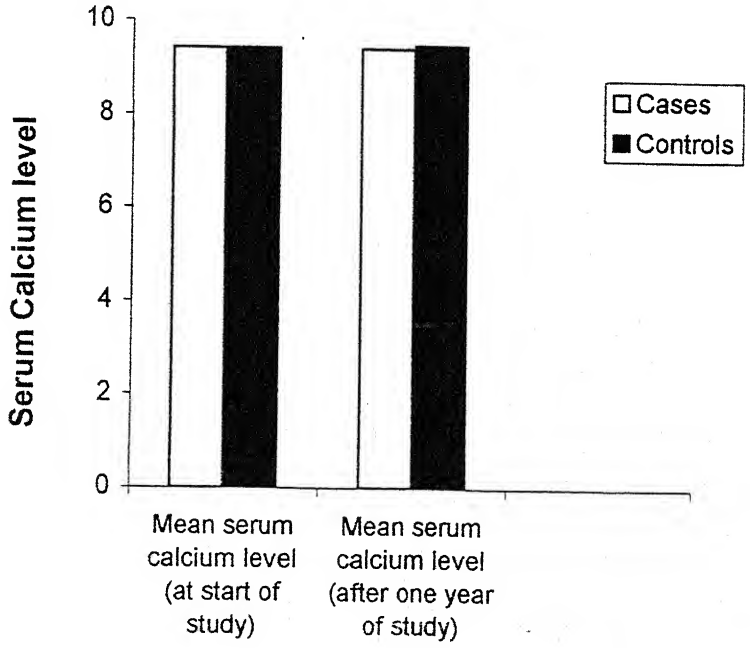

CHART NO.11

PREVENTION STUDY

12. AGE CHARACTERISTICS OF SUBJECTS UNDER STUDY :

Age Group (years)	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
31-40	6	24	6	24	12	24
41-50	16	64	16	64	32	64
51-60	3	12	3	12	6	12
61-70	0	0	0	0	0	0
Mean age	**44.3 ± 5.11 years**		**44.3 ± 5.11 years**			

13. DISTRIBUTION ACCORDING TO AGE OF ONSET OF MENOPAUSE :

Age Group (years)	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
31-40	16	64	7	28	23	46
41-50	9	36	16	64	25	50
51-60	0	-	2	8	2	4
61-70	0	-	0	0	0	0
Mean age	**39.1 ± 6.39 years**		**43.5 ± 5.28 years**			

PREVENTION STUDY

AGE CHARACTERISTICS OF SUBJECTS UNDER STUDY

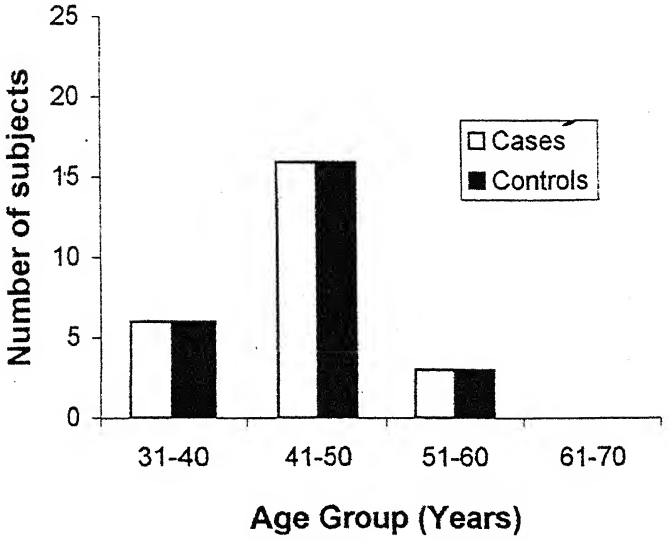

CHART NO. 12

DISTRIBUTION ACCORDING TO AGE OF ONSET OF MENOPAUSE

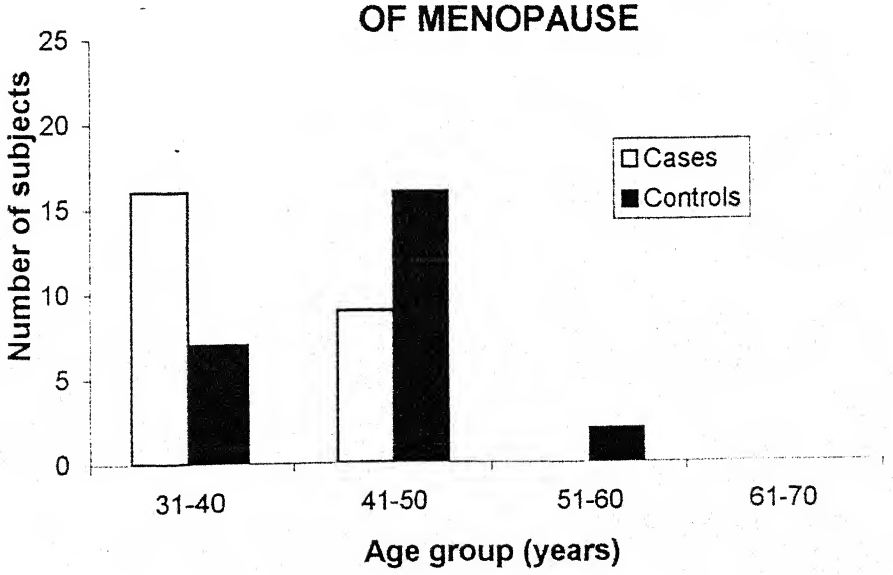

CHART NO.13

14. DISTRIBUTION ACCORDING TO DURATION OF MENOPAUSE :

Duration (years)	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
<1	6	24	8	32	14	28
1-5	18	72	16	64	34	68
6-10	1	4	1	4	2	4
11-15	0	0	0	0	0	0
Mean Duration	**3 ± 2.29 years**		**2.4 ± 2.4 years**			

15. DISTRIBUTION ACCORDING TO PARITY :

Parity	Cases		Controls		Total	
	n=25	%	n=25	%	n=50	%
P1	1	4	0	0	1	2
P2	4	16	3	12	7	14
P3	12	48	14	56	26	52
P4	8	32	8	32	16	32
Mean Duration	**3.08 ± 0.58 years**		**3.6 ± 0.63 years**			

DISTRIBUTION ACCORDING TO DURATION OF MENOPAUSE

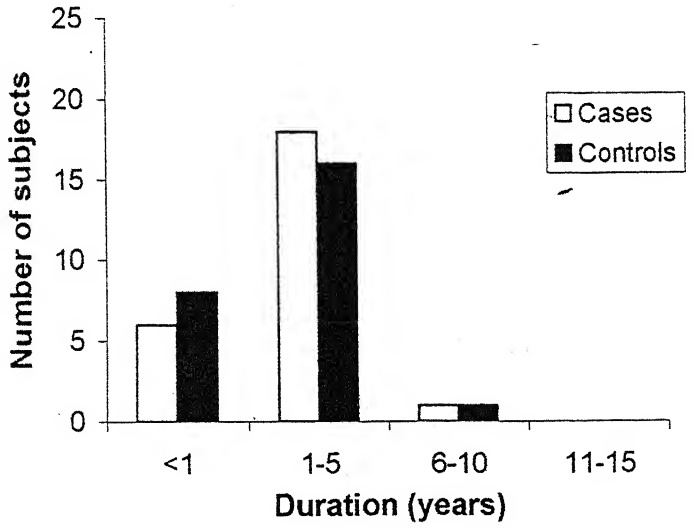

CHART NO.14

DISTRIBUTION ACCORDING TO PARITY

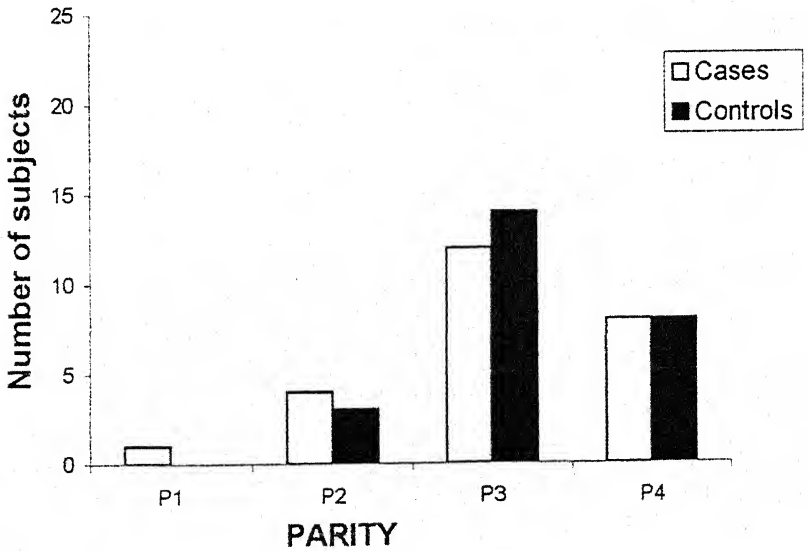

CHART NO.15

16. DISTRIBUTION ACCORDING TO TYPE OF MENOPAUSE :

Type	Cases		Controls	
	n=25	%		%
Natural	7	**28**	6	**24**
Surgical	18	**72**	19	**76**

17. DISTRIBUTION ACCORDING TO SOCIOECONOMIC STATUS :

Socioeconomic Status	Cases		Control		Total	
	n=25	%	n=25	%	n=50	%
Upper	0	0	0	0	0	0
Middle Upper	9	36	5	20	14	28
Middle Lower	**12**	**48**	**16**	**64**	**28**	**56**
Lower	4	16	4	16	8	16

DISTRIBUTION ACCORDING TO TYPE OF MENOPAUSE

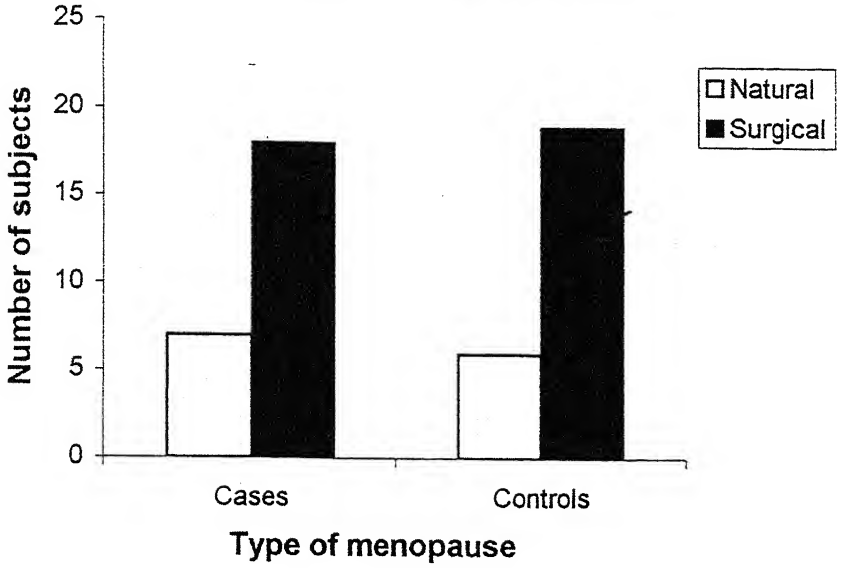

CHART NO.16

DISTRIBUTION ACCORDING TO SOCIOECONOMIC STATUS

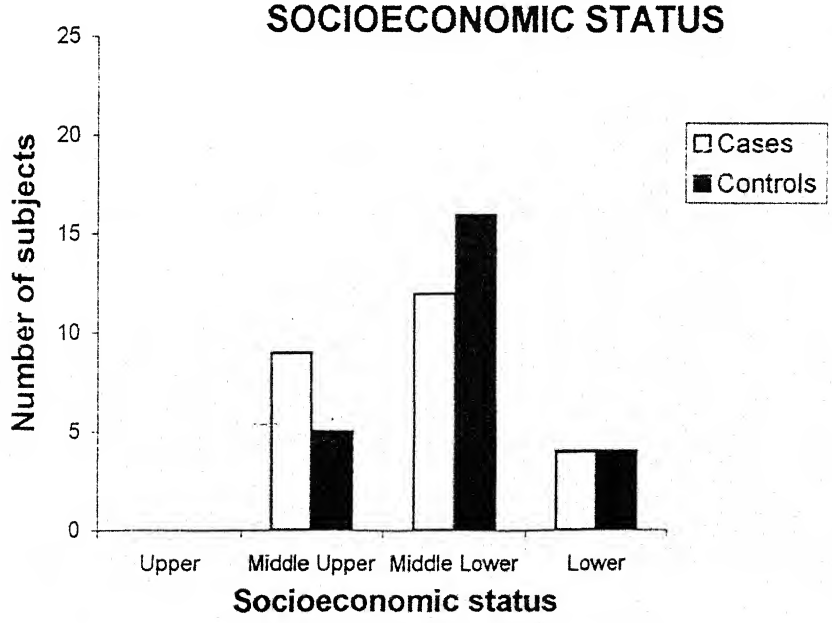

CHART NO. 17

18. DISTRIBUTION OF CHANGE IN MEAN BMD :

	Cases	Controls
Mean BMD at start of study	0.571	0.574
Change in BMD after One Year	(+) 0.007	(-) 0.002
Percent Change	**(+) 1.22 %**	**(-) 0.35 %**

19. DISTRIBUTION OF PERCENTAGE CHANGE IN BMD :

Percentage BMD Change	Cases		Control	
	n=25	% (+)	n=25	% (-)
0	6	**24**	13	**42**
≤1%	7	**2**	12	**48**
1.1-2%	12	**48**	0	**0**
>2%	0	**0**	0	**0**

DISTRIBUTION OF CHANGE IN MEAN BMD

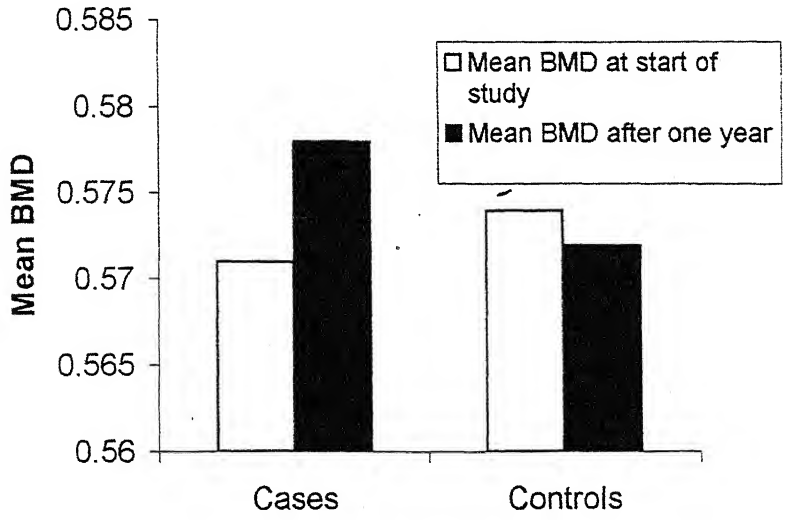

CHART NO.18

DISTRIBUTION OF PERCENTAGE CHANGE IN BMD

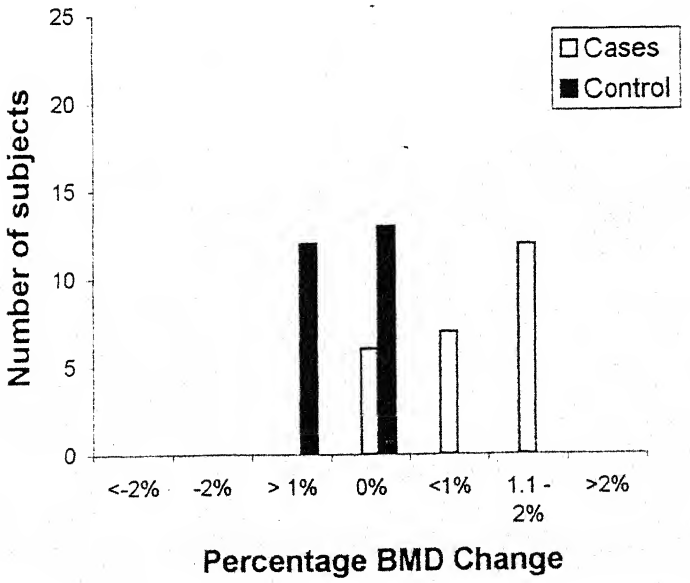

CHART NO.19

20. DISTRIBUTION OF MEAN BMD IN REFERENCE TO AGE :

Age group (years)	CASES	CONTROLS
	Mean BMD (at start of study)	Mean BMD (at start of study)
31-40	0.571	0.568
41-50	0.578	0.589
51-60	0.532	0.544
61-70	-	-

21. DISTRIBUTION OF MEAN BMD CHANGE IN STUDY POPULATION :

	Cases			Controls		
Age Group (years)	Mean BMD (at start of study)	Mean BMD (after one year)	**% Mean Change (+)**	Mean BMD (at start of study)	Mean BMD (after one year)	**% Mean Change (-)**
31-40	0.571	0.578	**1.2 %**	0.568	0.566	**0.35 %**
41-50	0.578	0.582	**0.7 %**	0.589	0.586	**0.51 %**
51-60	0.532	0.535	**0.7 %**	0.544	0.542	**0.37 %**
61-70	-	-	-	-	-	-

DISTRIBUTION OF MEAN BMD IN REFERENCE TO AGE

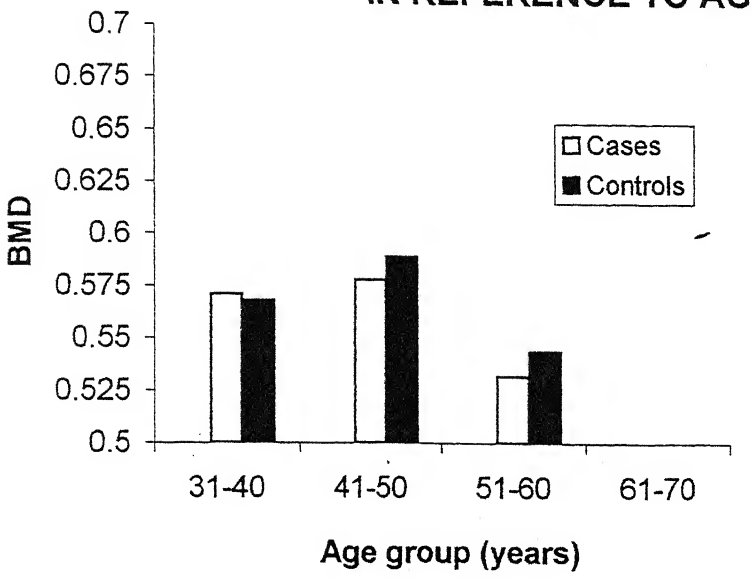

CHART NO.20

DISTRIBUTION OF MEAN BMD CHANGE IN STUDY POPULATION

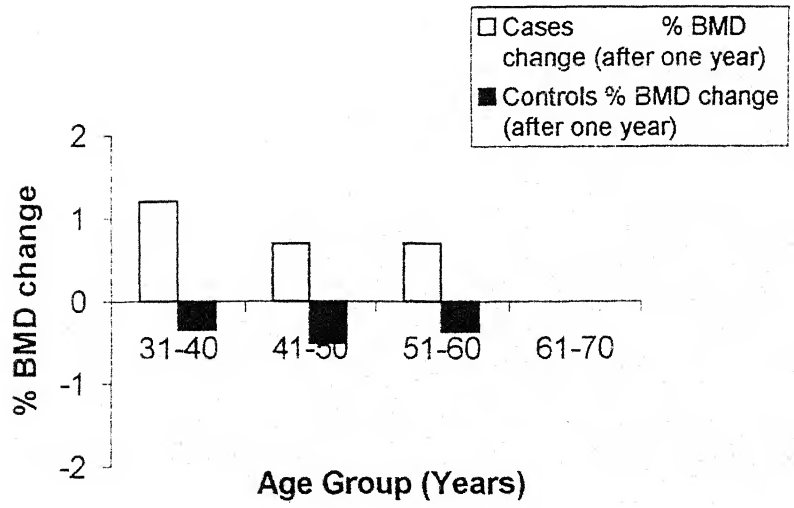

CHART NO.21

22. CHANGE IN MEAN SERUM CALCIUM VALUES :

	Cases	Controls
Mean serum calcium levels (at start of study)	9.46	9.46
Mean serum calcium levels (after one year)	9.47	9.47
Change in mean serum calcium levels	0.01	0.01
Percent change	**0**	**0**

CHANGE IN MEAN SERUM CALCIUM LEVEL

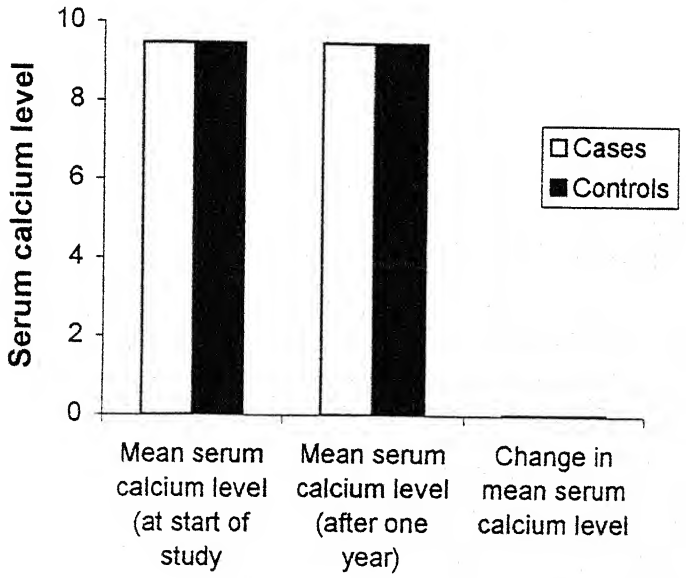

CHART NO. 22

23. INCIDENCE OF SIDE EFFECTS WITH RALOXIFENE THERAPY :

Side Effects	Cases (n=50)		Controls (n=50)	
	%	n	%	n
Hot Flushes (H)	**26**	**13**	**18**	**9**
Infection (I)	14	7	14	7
Abdominal pain (A)	6	3	8	4
Leg cramps (L)	**6**	**3**	**2**	**1**
Breast pain (B)	4	2	6	3
Chest pain (C)	4	2	2	1
Flatulence (F)	4	2	2	1
Dyspnea (D)	2	1	2	1

INCIDENCE OF SIDE EFFECTS WITH RALOXIFENE THERAPY

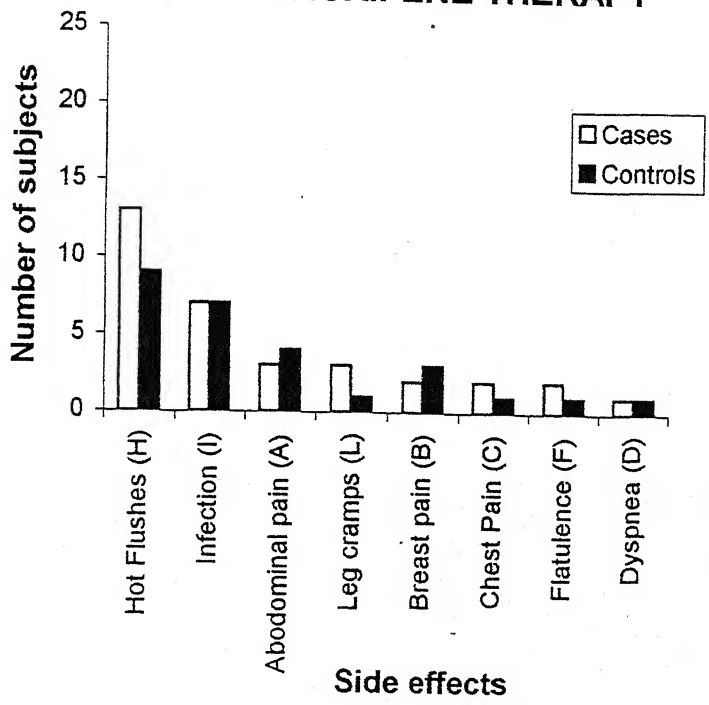

CHART NO. 23

DISCUSSION

Postmenopausal estrogen deficiency, though a physiological phenomenon causes much morbidity and is an indirect contributor to mortality. It has been proven beyond doubt that estrogen deficiency is the most significant contributor to accelerated osteoporosis in postmenopausal women. This stimulated the use of estrogen and related hormones for treating and preventing postmenopausal osteoporosis. Though effective, the use of estrogen was associated with significant side effects including increased incidence of endometrial carcinoma .To avoid such side effects, new molecules with estrogen agonist-antagonist properties were made. These were named Selective Estrogen Receptor Modulators (SERMs). Raloxifene is one such popular SERM. It acts as agonist at bone & CVS and antagonist in the endometrium. It offers benefits similar to estrogen but is devoid of serious side effects like endometrial carcinoma.

Our study is an effort to use the drug in Indian women for both treatment & prevention of postmenopausal osteoporosis and compare its benefits with the international trials. We have taken BMD & serum calcium as markers for the therapeutic effect of raloxifene and compared it with no hormonal therapy in our study.

The patients recruited for our study were taken from postmenopausal women attending the OPD and ward of Obstetrics &Gynaecology Department, M.L.B Medical College, Jhansi. The age of onset of menopause, duration of menopause, nature of menopause -whether natural or surgical, parity, socioeconomic status of the patients was noted.

The patient's BMD and serum calcium values were noted before starting the study. Based on the BMD values, the patients were initially classified as osteoporotic, osteopenic or normal based on BMD values<0.425gm/cm^2, 0.425-0.510gm/mcm^2 and >0.510gm/cm^2 respectively.

50 osteoporotic and osteopenic patients were included in the trial for treatment of osteporosis. Twenty-five women received raloxifene (60mg/day) and calcium (500 mg/day) whereas the control group of twenty five patients received only calcium supplement (500mg/day). The patients were distributed into trial & control groups randomly for all other variables.

50 postmenopausal women with normal BMD values were included in the study for use of raloxifene to prevent osteoporosis. Similar to the treatment study group, the prevention group was again divided into treated cases [60 mg. raloxifene/day + calcium 500 mg/day] and controls [calcium 500 mg/day only]. After one

year of therapy, the patients were evaluated for BMD & serum calcium values. The patients were also interviewed regularly for any side effects during the period of trial.

In the trial for treatment of postmenopausal osteoporosis, the treated population included 12 osteoporotic and 13 osteopenic but non-osteoporotic women. The mean age of these patients was 57.5 $\pm$ 5.27 years .The average BMD of this group of patients was 0.431 $\pm$ 0.041. After treatment for one year, the increase was + 0.0074 (1.71%). Change in BMD was observed in 80% of treated cases whereas 20% of those treated showed no change in BMD after one year of raloxifene therapy. The increase in BMD changed one patient from the osteopenic to normal and one patient from osteoporotic to osteopenic category.

In the non treated osteoporotic women, that is, the control group in the treatment of postmenopausal osteoporosis trial, 13 osteoporotic and 12 osteopenic patients received only calcium 500 mg/day supplement. The mean age of these subjects was 58.3 $\pm$ 5.45 years. The average BMD of this group was 0.434 $\pm$ 0.043. After one year, the BMD decreased by -0.0035 gm/cm^2 (0.80%).The decline in BMD was observed in15(60%) of patients whereas 10(40%) showed no decline in BMD over the one year

period. This decline in BMD changed one osteopenic patient into osteoporotic.

Ettinger et al 1999 conducted a 3 year, randomized trial in postmenopausal women with either moderate or severe vertebral fracture or those with femoral neck or lumbar spine BMD less than 2.5 SD of normal. The patients received raloxifene 30mg, 60mg, 150 mg/day or placebo. In the treated patients, the peak BMD was reached in 24 months of therapy. Raloxifene 60mg /day increased lumber spine BMD by 3.1% and femoral neck BMD by 1% over baseline compared to controls in whom lumbar spine BMD declined by 0.5% and femoral neck BMD declined by 1.2%.

Johnell et al in 1999 conducted a study on postmenopausal osteoporotic women with raloxifene, alendronate or placebo for 12 months. The increase in BMD in lumbar spine & femoral neck were 3.1% and1% for raloxifene 60mg/day; 4.3% and 2.7% for alendronate 10 mg/day; 5.3 % and 3.7% for raloxifene 60mg/day along with alendronate 10mg/day; and 0.1% and 0.3% with placebo. The difference in the effect produced by alendronate and raloxifene was not statistically significant. All active treatments increased BMD to a greater extent than placebo.

Lufkin et al 1998 treated osteoporotic women with raloxifene 60 mg/ day and 120 mg/day and treated controls with only calcium

and vitamin D. The mean age of women included was 67-69 years and duration of menopause 22 to 23.5 years. After 12 months of therapy, BMD in lumbar spine increased 1.8% and 2.1% in patients receiving raloxifene 60 mg/day and 120mg/day respectively. Ultradistal BMD increased 0.2% with raloxifene 60mg/day compared to controls, where it decreased by 2.7% over baseline. The effect was statistically significant.

In similar 2 year study by **Meunier et al 1999**, raloxifene 60 mg/day increased BMD in the lumbar spine, femoral neck, trochanter and total hip by 3.1%, 2.3%, 1.6% and 1.2% respectively, whereas in controls, it decreased by 0.1 %, 0.4%, 0.4% and 0.3% over baseline.

Clemett D.,Spencer M et al 2000 treated postmenopausal osteoporotic women with 60-150 mg/day of raloxifene. At the 60mg/day recommended doses , increases of 1.6-3.4%, 0.9-2.3% and 1-1.6% were reported in lumbar spine, femoral neck and total hip, respectively, versus <or =0.5% with placebo.

Hence the results of the present study are in accord with other International trials as summarized in the following table:-

Treatment Trials

	Mean BMD increase in treated postmenopausal women (cases)	Mean BMD fall in untreated postmenopausal women (controls)
Luskin 1998	1.8%	-2.7%
Ettinger 1999	1-1.6%	-<0.5%
Johnell 1999	1-3.1%	-0.3%
Meunier 1999	1.4-3.1%	-0.1-0.4%
Clemett 2000	1-2.3%	-<0.5%
Present study 2003	1.71%	-0.86%

In the trial for prevention of postmenopausal osteoporosis, the population selected included fifty (50) healthy women with normal bone mineral density. Of these, 25 women (50%) were randomly taken in the treated group. The mean age of these subjects was 44.3 $\pm$ 5.11 years. The mean bone mineral density was 0.571 $\pm$ 0.047. The mean increase in bone mineral density after one year was +0.007 gm/cm^2 (+1.22%). Bone mineral density increased in 19 treated women. No change in bone mineral density was seen in 6 of 25 women. The mean age of the subjects belonging to the control group of the prevention trails .was 44.3 $\pm$ 5.11 years. In the non treated women with normal BMD, the mean BMD was 0.574$\pm$ 0.048 and decline in BMD after one year was

0.002(-0.35%). Of these, 12 showed decline whereas 13 (56%) showed no change in BMD after one year.

In such a preventive study, **Delmas et al 1997** recruited 60 non-osteoporotic women. The mean age of subject was 55 years and mean duration of menopause 5.5 years. The patients received once daily raloxifene 30mg, 60mg or 150 mg or placebo oral once daily. Raloxifene consistently increased lumbar spine, femoral neck, total hip and total body BMD relative to baseline values; In contrast, BMD was generally reduced compared with baseline values in placebo group. Total body BMD increased in range of 1.2% -1.9% and declined in placebo group by 0.6%.

In a similar preventive trial by **Pavo et al 1999,** 98 patients with mean age 59 years and mean duration of menopause >/=2years received raloxifene 60mg/day Vs placebo. Raloxifene treated women showed increased BMD 1.1% in hip to 3.4% in lumber spine whereas the BMD of the untreated postmenopausai women declined by 0.6% in hip to 0.7% in femoral neck over the one year period.

Hence the results of the present study in assessing the efficacy of raloxifene in prevention of postmenopausal

osteoporosis are in accord with other international trials as summarized in the following table:-

Prevention trials

	Mean BMD increase in treated postmenopausal women (cases)	Mean BMD fall in untreated postmenopausal women (controls)
Delmas 1977	1.2%	--0.6%
Pavo 1999	1.1%	-0.6%
Present study 2003	1.16%	-0.35%

SUMMARY
&
CONCLUSION

This study was conducted to evaluate the efficacy of raloxifene, a selective estrogen receptor modulator, in prevention and treatment of postmenopausal osteoporosis. The postmenopausal women population included in the study were taken from those attending the outpatient and inpatient services of the Department of Obstetrics and Gynaecology at M.L.B. Medical College, Jhansi. Bone mineral density of these subjects was measured at the beginning of the study; and then, a year late, at the end of the study. Change in bone mineral density was the marker for change in osteoporosis status of the treated versus non-treated group.

The results of the study are summarized as under:-

1. Raloxifene is effective in preventing postmenopausal osteoporosis. Bone mineral density of subjects on regular raloxifene therapy increased by an average of 1.22% whereas it declined in controls by an average of 0.35%.

2. Raloxifene is effective in treating postmenopausal osteoporosis. Bone mineral density of postmenopausal osteopenic /osteoporotic women increased by an average of 1.71% whereas, there was a decrease in bone mineral density of 0.86% in untreated controls.

3. There was no significant difference in serum calcium values in treated and untreated subjects.

3. There was no significant difference in serum calcium values in treated and untreated subjects.

4. The most common side effect observed with raloxifene therapy was hot flushes. In no patient was the treatment stopped due to severe side effects.

BIBLIOGRAPHY

1. Macgregor JI, Jordan VC . Basic guide to the mechanisms of antiestrogen action. Pharmacol Rev 1998; 50: 151-96

2. Kauffman RF, Bryant HU, Yang N, et al. Preventing postmenopausal osteoporosis: an update on raloxifene. Drug News Perspect 1999; 12: 223-33

3. Khovidhunkit W, Shoback DM. Clinical effects of raloxifene hydrochloride in women. Ann Intern Med 1999; 130: 431-9

4. Yang NN , Venugopalan M, Hardikar S, et al. Identifiction of an estrogen response element activated by metabolites of 17- beta-estradiol and raloxifene. Science 1996; 273: 1222-5

5. Gize EA, Venugopalan M, Glasebrook AL, et al. Characterization of raloxifene binding and transactivtion properties off the estrogen receptor – beat (ERB) [abstractS428]. J Bone Miner Res 1997 12 Suppl.: S460

6. Yang NN, Bryant HU, Hardikar S, et al. Estrogen and raloxifene stimulate transforming growth factor-beta 3 gene expression in rat bone: a potential mechanism for estrogen-or raloxifene-mediated bone maintenance. Endocrinology 1996; 137: 2075- 84

7. Migliaccio S, Teti A, Taranta A, et al. Raloxifene modulates osteoclastogenesis and bone resorption in vitro [abstract].

Abstract of the 3rd International Symposium on Women's Health and Menopause; 1998 Jun 13-16, Florence:19

8. Migliaccio S, Taranta A, Teti A, et al. Raloxifene directly modulates bone cell activity in vitro [abstract]. Bone 1998; 23 Suppl.: 608

9. Lengner c, Green J, Bodine PVN, et al. Selective modulation of osteoblast growth and differentiation parameters by antiestrogens [abstract]. Bone 1998; 23 (5) Suppl.: S206

10. Delmas PD, Bjarnason NH, Mitlak B, et al. Effects of raloxifene on bone mineral density, serum cholesterol concentrations and uterine endometrium in postmenopausal women. N Engl J Med 1997; 337 (23) : 1641-7

11. Bjarnason NH, Delmas PD, Mitlak BH. Raloxifene maintains favourable effects on bone mineral density, bone turnover and serum lipids without endometrial stimulation in potmenopausal women 3-year study results [abstract Or22]. Osteoporos Int 1998; 8 Supl. 3: 11

12. Gunness M, Prestwood K, Lu Y, et al. Histomorphometric, bone marker and bone mineral density response to raloxifene HCl and premarin in postmenopausal women [abstract no.OR4-2]. The Endocrine Society 79th Annual Meeting Programme, 1997, Jun

11-14: Minneapolis, USA. The Endocrin Society press, Betheseda (MD): 67

13. Ettinger B, Black DM, Mitlak BH, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: results form a 3- year randomized clinical trial. JAMA1999; 282: 637- 45

14. Johnell O, Scheele W, Lu Y, et al. Effects of raloxifene (RLX), alendronate (ALN)and RLX+ALN on bone turnover in postmenopausal women with osteoporosis [abstract]. In: J Bone Miner Res 1999: 14 Suppl. 1: S157

15. LufkinEG, Whitaker MD, Nickelsen T, et al. Treatment of estalished postmenopausal osteoporosis with raloxifene: a randomized trial. J Bone Miner Res 1998; 13: 1747-54

16. Meunier PJ, Vignot E, Garnero P, et al. Treatment of postmenopausal women with osteoporosis or low bone density with raloxifene. Osteoporos Int 1999; 10 (4) : 330-6

17. Eli Lilly & Company. Evista: raloxifene hydrochloride prescribing information . Indianapolis (IN): Eli Lilly, 1999 Sep 30

18. Prvo I, Masanauskaite D, Rojinskaya L, et al. Effects of raloxifene (RLX) vs. placebo (PL) on bone mineral density (BMD) in postmenopausal women in the absence of calcium

supplementation [abstract]. J Bone Miner Res 1999: 14 Suppl. 1: S410

19. Davies GC , Huster WJ, Lu Y. Adverse events reported by postmenopausal women in controlled trials with raloxifene . Obstet Gynecol 1999; 93: 558- 65

20. Cohen FJ, Lu Y. Characterization of hot flashes reported by healthy postmenopausal women receiving raloxifene or placebo during osteoporosis prevention trials. Maturtas 2000; 34: 65-73

21. Thiebaud D, Secrest R.J. Selective estrogen receptor modulators: mechanism of action and clinical experience: Focus on raloxifene. Reprod Fertil Dev 2001. 13:331-336.

22. Mitlak BH, Cohen FJ. In searech of optimal long-term female hormone replacement: The potential of selective estrogen receptor modulators. Horm Res 1997; 48:155-163.

23. Bryant HU, Glasebrook AL, Yang NN, Sato M. A pharmacologic review of raloxifene. J Bone Miner Metab 1996; 14:1-9.

24. Black LJ, Jones CD, Falcone JF. Antagonism of estrogen action with a new benzothiophene derived antiestrogen. Life Sci 1983; 32L1031-1036.

25. Evans G, Bryant H. Sato M, et al. Raloxifene is a tissue-specific estrogen agonist. J Bone Miner Res 1993;81 Suppl. 11: 5134. A69.

26. Sato M. Glasebrook AL, Bryant HU. Raloxifene: a selective estrogen receptor modulator. J Bone Miner Metab 1994; 12 [Suppl 2]: 59-520.

27. Montano MM, Ekena K, Delage-Mourroux R, et al. An estrogen receptor-selective coregulator that potentiates the effectiveness of antiestrogens and represses the activity of estrogens. Proc Natl Acad Sci 1999;96[12]:6947-6952.

28. Jones PS, Parrott E, White INH. Activation of transcription by estrogen receptor alpha and beta is cell-type and promoter-dependent. J Biol Chem 1999;274[45]:32008-32014.

29. Giguere V, Tramblay A, Tremblay GB. Estrogen receptor β Re-evaluation of estrogen and antiestrogen signaling. Steroids 1998;63:335-339.

30. Kuiper GG, Gustafsson JA. The novel estrogen receptor-beta subtype: potential role in the cell-and promoter specific actions of estrogens and anti-estrogens. FEBS Lettreatment 1997;410:87-90.

31. Kuiper GG, Carlsson. B, Grandien K, et al. Comarison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. Endocrinology 1997;138:863-870.

32. Mosselman, S, Polman J, Dijkema R. ER-beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 1996;392:49-53.

33. Glasebrook AL, Phillipe DL, Sluka JP. Multiple binding sites for the anti-estrogen raloxifene. J Bone Miner Res 1993; 8 [Suppl. 1]:5268.

34. Brzozowaki AM, Pike ACW, Dauter Z, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. Nature 1997;389:753-758.

35. Gize EA, Venugopalan M, Glasebrook AL, Yang NN. Characterization of raloxifene binding and transactivation properties of the estrogen receptor-beta. J Bone Miner Res 1997;12[Suppl.1]:5428.

36. Grese TA, Sluka JP, Bryant HU, et al. Benzopyran selective estrogen receptor modulators [SERMS]: Pharmacological effects and structural correlation with raloxifene. Bioorg Med Chem Lett 1996;6:903-908.

37. Montano MM, Ekena K Krueger K, et al. Human estrogen receptor ligand activity inversion mutants: receptors that interpret antiestrogens as estrogens and estrogens as antiestrogens and discriminate among different antiestrogens. Mol Endocrinol 1996;10:230-242.

38. McDonnell DP. The molecular pharmacology of SERMs. Trends Endocrinol Metab 1999;10:301-311.

39. Yang NN, Venugopalan M, Hardikar S, Glasebrook A. Identification of an estrogen response element activated by metabolites of 17β-eatradiol and raloxifene. Science 1996;273[5279]:1222-1225.

40. Yang N, N, Bryant, H, Hardikar S, et al. Estrogen and raloxifene stimulate transforming growth factorβ gene expression in rat bone: a potential mechanism for estrogen-or raloxifene-mediated bone maintenance. Endocrinology 1966;137:2075-2084.

41. Eastell R, Adachi J, Harper K, et al. The effects of raloxifene on incident vertebral fractures in postmenopausal women with osteoporosis: 4-year results from the MORE trial. J Bone Min Res 2000;15[Suppl 1]:5229. Abstract 418.

42. Walsh BW, Kuller LH, Wild RA, et al. The effect of raloxifene on markers of cardiovascular risk in healthy, post-menopausal women. Atherosclerosis 1997;134[1,2]:182.

43. Walsh BW, Kuller LH, Wild RA et al Effects of raloxifene on serum lipids and coagulation factors in healthy postmenopausal women. JAMA 1998;279:1445-1451.

44. Walsh BW, Pauls, Wild RA, et al. The effects of hormone replacement therapy and raloxifene on C-reactive protein and coagulation factors in healthy postmenopausal women: A randomized, controlled trial. J Clin Endocrinol Metab 2000;85:214-218.

45. Barrett-Connor E, Grady D, Sashegyi A, et al. Raloxifene and cardiovascular events in osteoporotic postmenopausal women. Four year results from the MORE [Multiple Outcomes of Raloxifene Evaluation] Randomized Trial. JAMA 2002;287:847-857.

46. Kauffman RF, Bensch WR, Roudebush RE, et al. Hypocholesterolemic activity of raloxefene [LY 139481]: Pharmacological characterization as a selective estrogen receptor modulator. Pharmacol Exp Ther 1997;280:146-153.

47. Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. JAMA 1999;281:2189-2197.

48. Cauley J, Norton L, Lippman S, et al. Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. Breast Cancer Res Treatment 2001;65:125-134.

MASTER CHART

Serial no.	Age of patients (years)	Duration of Menopause (years)	Age of onset of menopause (years)	Type of menopause Natural(N) /Surgical(S)	Parity	Socio economic status	BMD (at start of study)	BMD (at end of study)	Serum Calcium (at start of study)	Serum Calcium (at end of study)	Side effects
1	34	<1	34	S	1	M-u	.621	.626	9.46	9.49	H
2	35	<1	34	S	4	M-u	.632	.637	9.58	9.60	D
3	36	<1	35	S	4	M-u	.527	.533	9.43	9.43	-
4	37	<1	36	S	4	M-u	.521	.528	9.34	9.36	H
5	38	<1	37	S	4	M-u	.597	.603	10.00	10.02	-
6	39	<1	38	S	4	M-u	.529	.535	9.52	9.52	F
7	52	4	48	N	3	M-u	.522	.522	9.63	9.62	I
8	53	7	46	N	2	M-u	.534	.547	9.81	9.84	H
9	52	2	50	N	2	M-u	.541	.547	9.10	9.11	I
10	41	3	38	S	2	M-l	.557	.563	9.15	9.15	C
11	42	4	38	S	2	M-l	.552	.562	9.72	9.74	H
12	43	5	38	S	4	M-l	.557	.562	9.82	9.85	I
13	44	5	39	S	4	M-l	.591	.598	9.23	9.23	I
14	45	5	40	N	4	M-l	.604	.612	9.46	9.47	B
15	46	4	42	S	3	M-l	.617	.617	9.31	9.33	-
16	47	3	44	S	3	M-l	.539	.539	9.38	9.37	H
17	48	2	46	N	3	M-l	.597	.597	9.50	9.51	-
18	49	2	47	N	3	M-l	.592	.592	9.58	9.59	L
19	50	3	47	N	3	M-l	.600	.600	9.68	9.71	-
20	41	4	37	S	3	M-l	.584	.599	9.16	9.18	H
21	42	3	39	S	3	M-l	.518	.523	9.20	9.20	A
22	43	3	40	S	3	L	.587	.593	9.24	9.24	A
23	43	2	41	S	3	L	.631	.636	9.39	9.40	H
24	42	2	40	S	3	L	.590	.597	9.36	9.36	-
25	44	4	40	S	3	L	.542	.550	9.48	9.50	-
26	32	<1	32	S	2	M-u	.520	.520	9.46	9.48	H
27	33	<1	32	S	3	M-l	.522	.519	9.21	9.23	-
28	34	<1	33	S	4	M-u	.528	.524	9.24	9.24	A
29	35	<1	34	S	3	M-l	.628	.628	9.29	9.30	B
30	36	<1	35	S	2	M-u	.610	.610	9.32	9.34	-
31	37	<1	36	S	3	M-l	.602	.597	9.42	9.41	H
32	57	7	50	N	3	M-l	.597	.597	9.68	9.68	-
33	58	3	55	N	3	M-l	.563	.563	9.66	9.66	I
34	59	4	53	N	4	M-u	.572	566	9.15	9.16	A
35	41	<1	40	S	3	M-l	.581	.581	9.19	9.21	-
36	42	5	41	S	2	M-l	.598	.595	9.20	9.20	H
37	43	3	42	S	3	M-u	.607	.607	10.05	9.99	I
38	44	3	41	S	4	M-l	.632	.628	9.86	9.86	-
39	45	4	41	S	3	M-l	.532	.527	9.76	9.77	I
40	46	2	44	S	3	L	531	531	9.48	9.49	-
41	47	3	44	S	4	M-l	548	544	9.57	9.60	H
42	48	4	44	S	3	L	576	576	9.41	9.41	L
43	49	3	46	S	3	M-l	552	546	9.34	9.33	-
44	50	4	46	N	4	L	583	583	9.38	9.38	I
45	49	3	46	N	3	M-l	.561	.558	9.36	9.35	-
46	48	2	46	N	4	L	.592	.592	9.50	9.51	C
47	47	3	44	S	3	M-l	.568	.568	9.58	9.58	-
48	46	4	42	S	4	M-l	.633	.628	9.60	9.61	F
49	45	3	42	S	3	M-l	.675	.675	9.43	9.44	-
50	44	5	41	S	4	M-l	.661	.655	9.58	9.54	H
51	43	3	40	S	4	L	.461	.461	9.10	9.12	H
52	46	4	42	S	4	L	.453	.453	9.11	9.13	-
53	49	5	44	S	4	L	.471	.471	9.24	9.24	-
54	70	15	55	N	4	L	.403	.403	9.30	9.31	H
55	69	14	55	N	4	L	.410	.410	9.39	9.38	-
56	68	15	53	N	4	L	.408	.418	9.40	9.40	B
57	67	10	57	N	4	L	.401	.412	9.50	9.50	-
58	66	11	55	S	4	L	.397	.405	9.48	9.49	H

Serial no.	Age of patients (years)	Duration of Menopause (years)	Age of onset of menopause (years)	Type of menopause Natural(N) /Surgical(S)	Parity	Socio economic status	BMD (at start of study)	BMD (at end of study)	Serum Calcium (at start of study)	Serum Calcium (at end of study)	Side effects
59	65	12	53	N	4	M-l	.393	.401	9.40	9.42	-
60	64	13	51	N	4	M-l	.400	.409	9.28	9.28	L
61	63	9	54	S	4	M-l	.399	.408	9.45	9.46	I
62	60	8	52	N	4	M-l	.401	.410	9.38	9.40	H
63	59	9	50	N	2	M-l	.438	.450	9.48	9.48	-
64	58	7	51	N	2	M-l	.427	.439	10.00	10.01	F
65	57	6	51	N	2	M-u	.493	.505	9.69	9.71	I
66	56	7	49	S	3	M-u	.481	.490	9.75	9.78	H
67	55	6	49	S	3	M-u	.462	.474	9.20	9.23	-
68	54	8	46	S	3	M-u	.459	.465	9.21	9.21	L
69	53	6	47	S	3	M-u	.432	.441	9.66	9.65	I
70	52	7	45	S	3	M-l	.500	.510	9.30	9.32	H
71	60	9	51	N	3	M-l	.401	.405	9.33	9.33	-
72	59	8	51	N	3	M-l	.410	.416	9.48	9.48	C
73	58	8	50	S	3	M-l	.422	.432	9.56	9.56	-
74	57	6	51	N	3	M-l	.431	.440	9.59	9.60	H
75	56	5	49	N	3	M-l	.429	.438	9.47	9.48	A
76	46	3	43	S	2	M-l	.502	.502	10.01	10.01	H
77	47	3	44	S	2	M-l	.493	.493	9.96	9.96	-
78	48	4	44	S	2	M-u	.489	.484	9.18	9.20	-
79	51	6	45	S	2	M-u	.472	.472	9.26	9.28	B
80	52	6	46	N	3	M-l	.461	.455	9.19	9.20	-
81	53	7	46	S	3	M-u	.450	.443	9.16	9.16	H
82	54	9	45	S	3	M-l	.491	.491	9.80	9.78	-
83	55	8	47	N	3	M-u	.440	.435	9.24	9.24	-
84	56	6	50	N	3	M-l	.430	.430	9.34	9.35	H
85	57	7	50	N	3	M-l	.412	.406	9.48	9.50	-
86	58	8	50	N	3	M-l	.411	.407	9.98	9.97	B
87	59	7	52	N	3	M-u	.433	.427	9.10	9.12	-
88	60	10	50	N	3	M-u	.446	.446	9.24	9.26	H
89	58	7	51	S	3	M-l	.453	.446	9.32	9.34	I
90	57	7	50	N	3	M-l	.401	.401	10.01	10.00	-
91	61	8	53	N	3	M-l	.410	.401	9.47	9.49	A
92	62	8	54	N	4	M-l	.420	.413	9.49	9.51	A
93	63	12	51	S	4	L	.422	.418	9.53	9.53	I
94	64	13	51	N	4	L	.403	.403	9.62	9.62	-
95	65	11	54	S	4	L	.415	.409	9.69	9.68	D
96	66	11	55	S	4	L	.400	.400	9.78	9.79	I
97	67	12	55	S	4	L	.402	.402	9.83	9.84	-
98	68	11	57	S	4	L	.397	.390	9.48	9.49	-
99	69	15	54	S	4	L	.396	.391	9.22	9.22	H
100	70	14	56	S	4	L	.390	.386	9.37	9.38	-

H= Hot flushes

I = Infection

A= Abdominal pain

L= Leg Cramps

B= Breast pain

C= Chest pain

F= Flatulence

D= Dyspnea